BioInitiative 2012

A Rationale for Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

BioInitiative Working Group 2012

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION I

Preface

Prepared for the BioInitiative Working Group
July 2007

PREFACE

The Organizing Committee thanks the participants of the BioInitiative Working Group for their integrity and intellectual courage in dealing with this controversial and important topic; and for devoting the time and energy to produce their chapters. The information and conclusions in each chapter are the responsibilities of the authors of that chapter.

The Group has produced what the authors hope will be a benchmark for good science and public health policy planning. It documents bioeffects, adverse health effects and public health conclusions about impacts of non-ionizing radiation (electromagnetic fields including extremely-low frequency ELF-EMF and radiofrequency/microwave or RF-EMF fields).

Societal decisions about this body of science have global implications. Good public health policy depends on acting soon enough, but not without cause, and with enough information to guide intelligent actions. To a great degree, it is the definition of the standard of evidence used to judge the scientific reports that shapes this debate. Disagreement about when the evidence is sufficient to take action has more to do with the outcome of various reviews and standard-setting proceedings than any other single factor. Whatever "standard of evidence" is selected to assess the strength of the science will deeply influence the outcome of decisions on public policy.

We are at a critical juncture in this world-wide debate. The answers lie not only in the various branches of science; but necessarily depend on the involvement of public health and policy professionals, the regulatory, legal and environmental protection sectors, and the public sector.

This has been a long-term collaboration of international scientists employing a multi-disciplinary approach to problem assessment and solving. Our work has necessarily relied on tools and approaches across the physical, biological and engineering sciences; and those of the environmental scientist and public health professional. Only when taken together can we see the whole and begin to take steps that can prevent possible harm and protect future generations.

Signed: David O Carpenter Signed: Cindy Sage

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SECTION I

Preface

Prepared for the BioInitiative Working Group
December 2012

PREFACE

Today, the BioInitiative 2012 Report updates five years of science, public health, public policy and global response to the growing health issue of chronic exposure to electromagnetic fields and radiofrequency radiation in the daily life of billions of people around the world.

The BioInitiative 2012 Report has been prepared by 29 authors from ten countries*, ten holding medical degrees (MDs), 21 PhDs, and three MsC, MA or MPHs. Among the authors are three former presidents of the Bioelectromagnetics Society, and five full members of BEMS. One distinguished author is the Chair of the Russian National Committee on Non-Ionizing Radiation. Another is a Senior Advisor to the European Environmental Agency. As in 2007, each author is responsible for their own chapter.

The great strength of the BioInitiative Report (www.bioinitiative.org) is that it has been done independent of governments, existing bodies and industry professional societies that have clung to old standards. Precisely because of this, the BioInitiative Report presents a solid scientific and public health policy assessment that is evidence-based.

The BioInitiative Report was first posted in August 2007. It still has a significant international viewing audience. Each year, about 1,000,000 people still visit the site. In the five years since it's publication, the BioInitiative website has been accessed over 10.5 million times, or four times every minute. Every five minutes on the average, a person somewhere in the world has logged on. More than 5.2 million files and 1 million pages of information has been downloaded. That is equivalent to more than 93,000 full copies of the 650+ page report (288.5 million kbytes).

The global conversation on why public safety limits for electromagnetic and radiofrequency fields remain thousands of time higher than exposure levels that health studies consistently show to be associated with serious health impacts has intensified since 2007. Roughly, 1800 new studies have been published in the last five years reporting effects at exposure levels ten to hundreds or thousands of times lower than allowed under safety limits in most countries of the world. Yet, no government has instituted comprehensive reforms. Some actions have been taken that highlight partial solutions. The Global Actions chapter presents milestone events that characterize the international 'sea change' of opinion that has taken place, and reports on precautionary advice and actions from around the world.

* Sweden (6), USA (10), India (2), Italy (2), Greece (2), Canada (2), Denmark (1), Austria (2), Slovac Republic (1), Russia (1)

The world's populations – from children to the general public to scientists and physicians – are increasingly faced with great pressures from advertising urging the incorporation of the latest wireless device into their everyday lives. This is occurring even while an elementary understanding the possible health consequences is beyond the ability of most people to grasp. The exposures are invisible, the testing meters are expensive and technically difficult to operate, the industry promotes new gadgets and generates massive advertising and lobbying campaigns that silence debate, and the reliable, non-wireless alternatives (like wired telephones and utility meters) are being discontinued against public will. There is little labeling, and little or no informed choice. In fact there is often not even the choice to stay with safer, wired solutions, as in the case of the 'smart grid' and smart wireless utility metering, an extreme example of a failed corporate-governmental partnership strategy, ostensibly for energy conservation.

A collision of the wireless technology rollout and the costs of choosing unwisely is beginning and will grow. The groundwork for this collision is being laid as a result of increased exposure, especially to radiofrequency fields, in education, in housing, in commerce, in communications and entertainment, in medical technologies and imaging, and in public and private transportation by air, bus, train and motor vehicles. Special concerns are the care of the fetus and newborn, the care for children with learning disabilities, and consideration of people under protections of the Americans With Disabilities Act, which includes people who have become sensitized and physiologically intolerant of chronic exposures. The 2012 Report now addresses these issues as well as presenting an update of issues previously discussed.

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SECTION 1

Summary for the Public

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Sage Associates, USA

Prepared for the BioInitiative Working Group

August 2007

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I. SUMMARY FOR THE PUBLIC

A. Introduction

You cannot see it, taste it or smell it, but it is one of the most pervasive environmental exposures in industrialized countries today. Electromagnetic radiation (EMR) or electromagnetic fields (EMFs) are the terms that broadly describe exposures created by the vast array of wired and wireless technologies that have altered the landscape of our lives in countless beneficial ways. However, these technologies were designed to maximize energy efficiency and convenience; not with biological effects on people in mind. Based on new studies, there is growing evidence among scientists and the public about possible health risks associated with these technologies.

Human beings are bioelectrical systems. Our hearts and brains are regulated by internal bioelectrical signals. Environmental exposures to artificial EMFs can interact with fundamental biological processes in the human body. In some cases, this can cause discomfort and disease. Since World War II, the background level of EMF from electrical sources has risen exponentially, most recently by the soaring popularity of wireless technologies such as cell phones (two billion and counting in 2006), cordless phones, WI-FI and WI-MAX networks. Several decades of international scientific research confirm that EMFs are biologically active in animals and in humans, which could have major public health consequences.

In today's world, everyone is exposed to two types of EMFs: (1) extremely low frequency electromagnetic fields (ELF) from electrical and electronic appliances and power lines and (2) radiofrequency radiation (RF) from wireless devices such as cell phones and cordless phones, cellular antennas and towers, and broadcast transmission towers. In this report we will use the term EMFs when referring to all electromagnetic fields in general; and the terms ELF and RF when referring to the specific type of exposure. They are both types of non-ionizing radiation, which means that they do not have sufficient energy to break off electrons from their orbits around atoms and ionize (charge) the atoms, as do x-rays, CT scans, and other forms of ionizing radiation. A glossary and definitions are provided in Section 18 to assist you. Some handy definitions you will probably need when reading about ELF and RF in this summary section (the language for measuring it) are shown with the references for this section.

B. Purpose of the Report

This report has been written by 14 (fourteen) scientists, public health and public policy experts to document the scientific evidence on electromagnetic fields. Another dozen outside reviewers have looked at and refined the Report.

The purpose of this report is to assess scientific evidence on health impacts from electromagnetic radiation below current public exposure limits and evaluate what changes in these limits are warranted now to reduce possible public health risks in the future.

Not everything is known yet about this subject; but what is clear is that the existing public safety standards limiting these radiation levels in nearly every country of the world look to be thousands of times too lenient. Changes are needed.

New approaches are needed to educate decision-makers and the public about sources of exposure and to find alternatives that do not pose the same level of possible health risks, while there is still time to make changes.

A working group composed of scientists, researchers and public health policy professionals (The BioInitiative Working Group) has joined together to document the information that must be considered in the international debate about the adequacy (or inadequacy) of existing public exposure standards.

This Report is the product of an international research and public policy initiative to give an overview of what is known of biological effects that occur at low-intensity EMFs exposures (for both radiofrequency radiation RF and power-frequency ELF, and various forms of combined exposures that are now known to be bioactive). The Report examines the research and current standards and finds that these standards are far from adequate to protect public health.

Recognizing that other bodies in the United States, United Kingdom, Australia, many European Union and eastern European countries as well as the World Health Organization are actively debating this topic, the BioInitiative Working Group has conducted a independent science and public health policy review process. The report presents solid science on this issue, and makes recommendations to decision-makers and the public. Conclusions of the individual authors, and overall conclusions are given in Table 2-1 (BioInitiative Overall Summary Chart).

Eleven (11) chapters that document key scientific studies and reviews identifying low-intensity effects of electromagnetic fields have been written by members of the BioInitiative Working Group. Section 16 and 17 have been prepared by public health and policy experts. These sections discusses the standard of evidence which should be applied in public health planning, how the scientific information should be evaluated in the context of prudent public health policy, and identifies the basis for taking precautionary and preventative actions that are proportionate to the knowledge at hand. They also evaluate the evidence for ELF that leads to a recommendation for new public safety limits (not precautionary or preventative actions, as need is demonstrated).

Other scientific review bodies and agencies have reached different conclusions than we have by adopting standards of evidence so unreasonably high as to exclude any conclusions likely to lead to new public safety limits. Some groups are actually recommending a relaxation of the existing (and inadequate) standards. Why is this happening? One reason is that exposure limits for ELF and RF are developed by bodies of scientists and engineers that belong to professional societies who have traditionally developed recommendations; and then government agencies have adopted those recommendations. The standard-setting processes have little, if any, input from other stakeholders outside professional engineering and closely-related commercial interests. Often, the industry view of allowable risk and proof of harm is most influential, rather than what public health experts would determine is acceptable.

Main Reasons for Disagreement among Experts

1) Scientists and public health policy experts use very different definitions of the standard of evidence used to judge the science, so they come to different conclusions about what to do. Scientists do have a role, but it is not exclusive and other opinions matter.
2) We are all talking about essentially the same scientific studies, but use a different way of measuring when "enough is enough" or "proof exists".
3) Some experts keep saying that all studies have to be consistent (turn out the same way every time) before they are comfortable saying an effect exists.
4) Some experts think that it is enough to look only at short-term, acute effects.
5) Other experts say that it is imperative we have studies over longer time (showing the effects of chronic exposures) since that is what kind of world we live in.
6) Some experts say that everyone, including the very young, the elderly, pregnant women, and people with illnesses have to be considered – others say only the average person (or in the case of RF, a six-foot tall man) matter.
7) There is no unexposed population, making it harder to see increased risk of diseases.
8) The lack of consensus about a single biological mechanism of action.
9) The strength of human epidemiological studies reporting risks from ELF and RF exposures, but animal studies don't show a strong toxic effect.
10) Vested interests have a substantial influence on the health debate.

Public Policy Decisions

Safety limits for public exposure to EMFs need to be developed on the basis of interaction among not only scientists, but also public health experts, public policy makers and the general public.

"In principle, the assessment of the evidence should combine with judgment based on other societal values, for example, costs and benefits, acceptability of risks, cultural preferences, etc. and result in sound and effective decision-making. Decisions on these matters are eventually taken as a function of the views, values and interests of the stakeholders participating in the process, whose opinions are then weighed depending on several factors. Scientific evidence perhaps carries, or should carry, relatively heavy weight, but grants no exclusive status; decisions will be evidence-based but will also be based on other factors." (1)

The clear consensus of the BioInitiative Working Group members is that the existing public safety limits are inadequate for both ELF and RF.

These proposals reflect the evidence that a positive assertion of safety with respect to chronic exposure to low-intensity levels of ELF and RF cannot be made. As with many other standards for environmental exposures, these proposed limits may not be totally protective, but more stringent standards are not realistic at the present time. Even a small increased risk for cancer and neurodegenerative diseases translates into an enormous public health consequence. Regulatory action for ELF and preventative actions for RF are warranted at this time to reduce exposures and inform the public of the potential for increased risk; at what levels of chronic exposure these risks may be present; and what measures may be taken to reduce risks.

C. Problems with Existing Public Health Standards (Safety Limits)

Today's public exposure limits for telecommunications are based on the presumption that heating of tissue (for RF) or induced electric currents in the body (for ELF) are the only concerns when living organisms are exposed to RF. These exposures can create tissue heating that is well known to be harmful in even very short-term doses. As such, thermal limits do serve a purpose. For example, for people whose occupations require them to work around radar facilities or RF heat-sealers, or for people who install and service wireless antenna tower, thermally-based limits are necessary to prevent damage from heating (or, in the case of power-frequency ELF from induced current flow in tissues). In the past, scientists and engineers developed exposure standards for electromagnetic radiation based what we now believe are faulty assumptions that the right way to measure how much non-ionizing energy humans can tolerate (how much exposure) without harm is to measure only the heating of tissue (RF) or induced currents in the body (ELF).

In the last few decades, it has been established beyond any reasonable doubt that bioeffects and some adverse health effects occur at far lower levels of RF and ELF exposure where no heating (or induced currents) occurs at all; some effects are shown to occur at several hundred thousand times below the existing public safety limits where heating is an impossibility.

It appears it is the INFORMATION conveyed by electromagnetic radiation (rather than heat) that causes biological changes - some of these biological changes may lead to loss of wellbeing, disease and even death.

Effects occur at non-thermal or low-intensity exposure levels thousands of times below the levels that federal agencies say should keep the public safe. For many new devices operating with wireless technologies, the devices are exempt from any regulatory standards. The existing standards have been proven to be inadequate to control against harm from low-intensity, chronic exposures, based on any reasonable, independent assessment of the scientific literature. It means that an entirely new basis (a biological basis) for new exposure standards is needed. New standards need to take into account what we have learned about the effects of ELF and RF (all non-ionizing electromagnetic radiation and to design new limits based on biologically-demonstrated effects that are important to proper biological function in living organisms. It is vital to do so because the explosion of new sources has created unprecedented levels of artificial electromagnetic fields that now cover all but remote areas of the habitable space on earth. Mid-course corrections are needed in the way we accept, test and deploy new technologies that expose us to ELF and RF in order to avert public health problems of a global nature.

Recent opinions by experts have documented deficiencies in current exposure standards. There is widespread discussion that thermal limits are outdated, and that biologically-based exposure standards are needed. Section 4 describes concerns expressed by WHO, 2007 in its ELF Health Criteria Monograph; the SCENIHR Report, 2006 prepared for the European Commission; the UK SAGE Report, 2007; the Health Protection Agency, United Kingdom in 2005; the NATO Advanced Research Workshop in 2005; the US Radiofrequency Interagency Working Group in 1999; the US Food and Drug Administration in 2000 and 2007; the World Health Organization in 2002; the International Agency for Cancer Research (IARC, 2001), the United Kingdom Parliament Independent Expert Group Report on Mobile Phones – Stewart Report, 2000) and others.

A pioneer researcher, the late Dr. Ross Adey, in his last publication in Bioelectromagnetic Medicine (P. Roche and M. Markov, eds. 2004) concluded:

> "*There are major unanswered questions about possible health risks that may arise from exposures to various man-made electromagnetic fields where these human exposures are intermittent, recurrent, and may extend over a significant portion of the lifetime of the individual.*"
>
> *"Epidemiological studies have evaluated ELF and radiofrequency fields as possible risk factors for human health, with historical evidence relating rising risks of such factors as progressive rural electrification, and more recently, to methods of electrical power distribution and utilization in commercial buildings. Appropriate models describing these bioeffects are based in non-equilibrium thermodynamics, with nonlinear electrodynamics as an integral feature. Heating models, based in equilibrium thermodynamics, fail to explain an impressive new frontier of much greater significance. ….. Though incompletely understood, tissue free radical interactions with magnetic fields may extend to zero field levels." (2)*

There may be no lower limit at which exposures do not affect us. Until we know if there is a lower limit below which bioeffects and adverse health impacts do not occur, it is unwise from a public health perspective to continue "business-as-usual" deploying new technologies that increase ELF and RF exposures, particularly involuntary exposures.

II. SUMMARY OF THE SCIENCE

A. Evidence for Cancer

1. Childhood Leukemia

The evidence that power lines and other sources of ELF are consistently associated with higher rates of childhood leukemia has resulted in the International Agency for Cancer Research (an arm of the World Health Organization) to classify ELF as a Possible Human Carcinogen (in the Group 2B carcinogen list). Leukemia is the most common type of cancer in children.

There is little doubt that exposure to ELF causes childhood leukemia.

The exposure levels for increased risk are quite low – just above background or ambient levels and much lower than current exposure limits. The existing ICNIRP limit is 1000 mG (904 mG in the US) for ELF. Increased risk for childhood leukemia starts at levels almost one thousand times below the safety standard. Leukemia risks for young boys are reported in one study to double at only 1.4 mG and above (7) Most other studies combine older children with younger children (0 to 16 years) so that risk levels do not reach statistical significance until exposure levels reach 2 mG or 3 mG. Although some reviews have combined studies of childhood leukemia in ways that indicate the risk level starts at 4 mG and above; this does not reflect many of the studies reporting elevated risks at the lower exposure levels of 2 mG and 3 mG.

2. Other Childhood Cancers

Other childhood cancers have been studied, including brain tumors, but not enough work has been done to know if there are risks, how high these risks might be or what exposure levels might be associated with increased risks. The lack of certainty about other childhood cancers should not be taken to signal the "all clear"; rather it is a lack of study.

The World Health Organization ELF Health Criteria Monograph No 322 (2007) says that other childhood cancers "cannot be ruled out". (8)

There is some evidence that other childhood cancers may be related to ELF exposure but not enough studies have been done.

Several recent studies provide even stronger evidence that ELF is a risk factor for childhood leukemia and cancers later in life. In the first study (9), children who were recovering in high-ELF environments had poorer survival rates (a 450% increased risk of dying if the ELF fields were 3 mG and above). In the second study, children who were recovering in 2 mG and above ELF environments were 300% more likely to die than children exposed to 1 mG and below. In this second study, children recovering in ELF environments between 1 and 2 mG also had poorer survival rates, where the increased risk of dying was 280%. (10) These two studies give powerful new information that ELF exposures in children can be harmful at levels above even 1 mG. The third study looked what risks for cancer a child would have later in life, if that child was raised in a home within 300 meters of a high-voltage electric power line. (11) For children who were raised for their first five years of life within 300 meters, they have a life-time risk that is 500% higher for developing some kinds of cancers.

Children who have leukemia and are in recovery have poorer survival rates if their ELF exposure at home (or where they are recovering) is between 1mG and 2 mG in one study; over 3 mG in another study.

Given the extensive study of childhood leukemia risks associated with ELF, and the relatively consistent findings that exposures in the 2 mG to 4 mG range are associated with increased risk to children, a 1 mG limit for habitable space is recommended for new construction. While it is difficult and expensive to retrofit existing habitable space to a 1 mG level, and is also recommended as a desirable target for existing residences and places where children and pregnant women may spend prolonged periods of time.

New ELF public exposure limits are warranted at this time, given the existing scientific evidence and need for public health policy intervention and prevention.

3. *Brain Tumors and Acoustic Neuromas*

Radiofrequency radiation from cell phone and cordless phone exposure has been linked in more than one dozen studies to increased risk for brain tumors and/or acoustic neuromas (a tumor in the brain on a nerve related to our hearing).

People who have used a cell phone for ten years or more have higher rates of malignant brain tumor and acoustic neuromas. It is worse if the cell phone has been used primarily on one side of the head.

For brain tumors, people who have used a cell phone for 10 years or longer have a 20% increase in risk (when the cell phone is used on both sides of the head). For people who have used a cell phone for 10 years or longer predominantly on one side of the head, there is a 200% increased risk of a brain tumor. This information relies on the combined results of many brain tumor/cell phone studies taken together (a meta-analysis of studies).

People who have used a cordless phone for ten years or more have higher rates of malignant brain tumor and acoustic neuromas. It is worse if the cordless phone has been used primarily on one side of the head.

The risk of brain tumor (high-grade malignant glioma) from cordless phone use is 220% higher (both sides of the head). The risk from use of a cordless phone is 470% higher when used mostly on only one side of the head.

For acoustic neuromas, there is a 30% increased risk with cell phone use at ten years and longer; and a 240% increased risk of acoustic neuroma when the cell phone is used mainly on one side of the head. These risks are based on the combined results of several studies (a meta-analysis of studies).

For use of cordless phones, the increased risk of acoustic neuroma is three-fold higher (310%) when the phone is mainly used on one side of the head.

The current standard for exposure to the emissions of cell phones and cordless phones is not safe considering studies reporting long-term brain tumor and acoustic neuroma risks.

Other indications that radiofrequency radiation can cause brain tumors comes from exposures to low-level RF other than from cell phone or cordless phone use. Studies of people who are exposed in their work (occupational exposure) show higher brain tumor rates as well. Kheifets (1995) reported a 10% to 20% increased risk of brain cancer for those employed in electrical occupations. This meta-analysis surveyed 29 published studies of brain cancer in relation to occupational EMFs exposure or work in electrical occupations. (6). The evidence for a link between other sources of RF exposure like working at a job with EMFs exposure is consistent with a moderately elevated risk of developing brain tumors.

4. *Other Adult Cancers*

There are multiple studies that show statistically significant relationships between occupational exposure and leukemia in adults (see Chapter 11), in spite of major limitations in the exposure assessment. A very recent study by Lowenthal et al. (2007) investigated leukemia in adults in relation to residence near to high-voltage power lines. While they found elevated risk in all adults living near to the high voltage power lines, they found an OR of 3.23 (95% CI = 1.26-8.29) for individuals who spent the first 15 years of life within 300 m of the power line. This study provides support for two important conclusions: adult leukemia is also associated with EMF exposure, and exposure during childhood increases risk of adult disease.

A significant excess risk for adult brain tumors in electrical workers and those adults with occupational EMF exposure was reported in a meta-analysis (review of many individual studies) by Kheifets et al., (1995). This is about the same size risk for lung cancer and secondhand smoke (US DHHS, 2006). A total of 29 studies with populations from 12 countries were included in this meta-analysis. The relative risk was reported as 1.16 (CI = 1.08 – 1.24) or a 16% increased risk for all brain tumors. For gliomas, the risk estimate was reported to be 1.39 (1.07 – 1.82) or a 39% increased risk for those in electrical occupations. A second meta-analysis published by Kheifets et al., ((2001) added results of 9 new studies published after 1995. It reported a new pooled estimate (OR = 1.16, 1.08 – 1.01) that showed little change in the risk estimate overall from 1995.

The evidence for a relationship between exposure and breast cancer is relatively strong in men (Erren, 2001), and some (by no means all) studies show female breast cancer also to be elevated with increased exposure (see Chapter 12). Brain tumors and acoustic neuromas are more common in exposed persons (see Chapter 10). There is less published evidence on other cancers, but Charles et al. (2003) report that workers in the highest 10% category for EMF exposure were twice as likely to die of prostate cancer as those exposed at lower levels (OR 2.02, 95% CI = 1.34-3.04). Villeneuve et al. (2000) report statistically significant elevations of non-Hodgkin's lymphoma in electric utility workers in relation to EMF exposure, while Tynes et al. (2003) report elevated rates of malignant melanoma in persons living near to high voltage power lines. While these observations need replication, they suggest a relationship between exposure and cancer in adults beyond leukemia.

In total the scientific evidence for adult disease associated with EMF exposure is sufficiently strong for adult cancers that preventive steps are appropriate, even if not all reports have shown exactly the same positive relationship. This is especially true since many factors reduce our ability to see disease patterns that might be related to EMF exposure: there is no unexposed population for comparison, for example, and other difficulties in exposure assessment, The evidence for a relationship between EMF exposure and adult cancers and neurodegenerative diseases is sufficiently strong at present to merit preventive actions to reduce EMF exposure.

5. Breast Cancer

There is rather strong evidence from multiple areas of scientific investigation that ELF is related to breast cancer. Over the last two decades there have been numerous epidemiological studies (studies of human illness) on breast cancer in both men and women, although this relationship remains controversial among scientists. Many of these studies report that ELF exposures are related to increased risk of breast cancer (not all studies report such effects, but then, we do not expect 100% or even 50% consistency in results in science, and do not require it to take reasonable preventative action).

The evidence from studies on women in the workplace rather strongly suggests that ELF is a risk factor for breast cancer for women with long-term exposures of 10 mG and higher.

Breast cancer studies of people who work in relatively high ELF exposures (10 mG and above) show higher rates of this disease. Most studies of workers who are exposed to ELF have defined high exposure levels to be somewhere between 2 mG and 10 mG; however this kind of mixing of relatively low to relatively high ELF exposure just acts to dilute out real risk levels. Many of the occupational studies group exposures so that the highest group is exposed to 4 mG and above. What this means is that a) few people are exposed to much higher levels and b) illness patterns show up at relatively low ELF levels of 4 mG and above. This is another way of demonstrating that existing ELF limits that are set at 933-1000 mG are irrelevant to the exposure levels reporting increased risks.

Laboratory studies that examine human breast cancer cells have shown that ELF exposure between 6 mG and 12 mG can interfere with protective effects of melatonin that fights the growth of these breast cancer cells. For a decade, there has been evidence that human breast cancer cells grow faster if exposed to ELF at low environmental levels. This is thought to be because ELF exposure can reduce melatonin levels in the body. The presence of melatonin in breast cancer cell cultures is known to reduce the growth of cancer cells. The absence of melatonin (because of ELF exposure or other reasons) is known to result in more cancer cell growth.

Laboratory studies of animals that have breast cancer tumors have been shown to have more tumors and larger tumors when exposed to ELF and a chemical tumor promoter at the same time. These studies taken together indicate that ELF is a likely risk factor for breast cancer, and that ELF levels of importance are no higher than many people are exposed to at home and at work. A reasonable suspicion of risk exists and is sufficient evidence on which to recommend new ELF limits; and to warrant preventative action.

Given the very high lifetime risks for developing breast cancer, and the critical importance of prevention; ELF exposures should be reduced for all people who are in high ELF environments for prolonged periods of time.

Reducing ELF exposure is particularly important for people who have breast cancer. The recovery environment should have low ELF levels given the evidence for poorer survival rates for childhood leukemia patients in ELF fields over 2 mG or 3 mG. Preventative action for those who may be at higher risk for breast cancer is also warranted (particularly for those taking tamoxifen as a way to reduce the risk of getting breast cancer, since in addition to reducing the effectiveness of melatonin, ELF exposure may also reduce the effectiveness of tamoxifen at these same low exposure levels). There is no excuse for ignoring the substantial body of evidence we already have that supports an association between breast cancer and ELF exposure; waiting for conclusive evidence is untenable given the enormous costs and societal and personal burdens caused by this disease.

Studies of human breast cancer cells and some animal studies show that ELF is likely to be a risk factor for breast cancer. There is supporting evidence for a link between breast cancer and exposure to ELF that comes from cell and animal studies, as well as studies of human breast cancers.

These are just some of the cancer issues to discuss. It may be reasonable now to make the assumption that all cancers, and other disease endpoints might be related to, or worsened by exposures to EMFs (both ELF and RF).

If one or more cancers are related, why would not all cancer risks be at issue? It can no longer be said that the current state of knowledge rules out or precludes risks to human health. The enormous societal costs and impacts on human suffering by not dealing proactively with this issue require substantive public health policy actions; and actions of governmental agencies charged with the protection of public health to act on the basis of the evidence at hand.

B. Changes in the Nervous System and Brain Function

Exposure to electromagnetic fields has been studies in connection with Alzheimer's disease, motor neuron disease and Parkinson's disease. (4) These diseases all involve the death of specific neurons and may be classified as neurodegenerative diseases. There is evidence that high levels of amyloid beta are a risk factor for Alzheimer's disease, and exposure to ELF can increase this substance in the brain. There is considerable evidence that melatonin can protect the brain against damage leading to Alzheimer's disease, and also strong evidence that exposure to ELF can reduce melatonin levels. Thus it is hypothesized that one of the body's main protections against developing Alzheimer's disease (melatonin) is less available to the body when people are exposed to ELF. Prolonged exposure to ELF fields could alter calcium (Ca2+) levels in neurons and induce oxidative stress (4). It is also possible that prolonged exposure to ELF fields may stimulate neurons (particularly large motor neurons) into synchronous firing, leading to damage by the buildup of toxins.

Evidence for a relationship between exposure and the neurodegenerative diseases, Alzheimer's and amyotrophic lateral sclerosis (ALS), is strong and relatively consistent (see Chapter 12). While not every publication shows a statistically significant relationship between exposure and disease, ORs of 2.3 (95% CI = 1.0-5.1 in Qio et al., 2004), of 2.3 (95% CI = 1.6-3.3 in Feychting et al., 2003) and of 4.0 (95% CI = 1.4-11.7 in Hakansson et al., 2003) for Alzheimer's Disease, and of 3.1 (95% CI = 1.0-9.8 in Savitz et al., 1998) and 2.2 (95% CI = 1.0-4.7 in Hakansson et al., 2003) for ALS cannot be simply ignored.

Alzheimer's disease is a disease of the nervous system. There is strong evidence that long-term exposure to ELF is a risk factor for Alzheimer's disease.

Concern has also been raised that humans with epileptic disorders could be more susceptible to RF exposure. Low-level RF exposure may be a stressor based on similarities of neurological effects to other known stressors; low-level RF activates both endogenous opioids and other substances in the brain that function in a similar manner to psychoactive drug actions. Such effects in laboratory animals mimic the effects of drugs on the part of the brain that is involved in addiction.

Laboratory studies show that the nervous system of both humans and animals is sensitive to ELF and RF. Measurable changes in brain function and behavior occur at levels associated with new technologies including cell phone use. Exposing humans to cell phone radiation can change brainwave activity at levels as low as 0.1 watt per kilogram SAR (W/Kg)*** in comparison to the US allowable level of 1.6 W/Kg and the International Commission for Non-ionizing Radiation Protection (ICNIRP) allowable level of 2.0 W/Kg. It can affect memory and learning. It can affect normal brainwave activity. ELF and RF exposures at low levels are able to change behavior in animals.

There is little doubt that electromagnetic fields emitted by cell phones and cell phone use affect electrical activity of the brain.

Effects on brain function seem to depend in some cases on the mental load of the subject during exposure (the brain is less able to do two jobs well simultaneously when the same part of the brain is involved in both tasks). Some studies show that cell phone exposure speeds up the brain's activity level; but also that the efficiency and judgment of the brain are diminished at the same time. One study reported that teenage drivers had slowed responses when driving and exposed to cell phone radiation, comparable to response times of elderly people. Faster thinking does not necessarily mean better quality thinking.

Changes in the way in which the brain and nervous system react depend very much on the specific exposures. Most studies only look at short-term effects, so the long-term consequences of exposures are not known.

Factors that determine effects can depend on head shape and size, the location, size and shape of internal brain structures, thinness of the head and face, hydration of tissues, thickness of various tissues, dialectric constant of the tissues and so on. Age of the individual and state of health also appear to be important variables. Exposure conditions also greatly influence the outcome of studies, and can have opposite results depending on the conditions of exposure including frequency, waveform, orientation of exposure, duration of exposure, number of exposures, any pulse modulation of the signal, and when effects are measured (some responses to RF are delayed). There is large variability in the results of ELF and RF testing, which would be expected based on the large variability of factors that can influence test results. However, it is clearly demonstrated that under some conditions of exposure, the brain and nervous system functions of humans are altered. The consequence of long-term or prolonged exposures have not been thoroughly studied in either adults or in children.

The consequence of prolonged exposures to children, whose nervous systems continue to develop until late adolescence, is unknown at this time. This could have serious implications to adult health and functioning in society if years of exposure of the young to both ELF and RF result in diminished capacity for thinking, judgment, memory, learning, and control over behavior.

People who are chronically exposed to low-level wireless antenna emissions report symptoms such as problems in sleeping (insomnia), fatigue, headache, dizziness, grogginess, lack of concentration, memory problems, ringing in the ears (tinnitus), problems with balance and orientation, and difficulty in multi-tasking. In children, exposures to cell phone radiation have resulted in changes in brain oscillatory activity during some memory tasks. Although scientific studies as yet have not been able to confirm a cause-and-effect relationship; these complaints are widespread and the cause of significant public concern in some countries where wireless technologies are fairly mature and widely distributed (Sweden, Denmark, France, Germany, Italy, Switzerland, Austria, Greece, Israel). For example, the roll-out of the new 3rd Generation wireless phones (and related community-wide antenna RF emissions in the Netherlands) caused almost immediate public complaints of illness.(5)

Conflicting results from those few studies that have been conducted may be based on the difficulty in providing non-exposed environments for testing to compare to environments that are intentionally exposed. People traveling to laboratories for testing are pre-exposed to a multitude of RF and ELF exposures, so they may already be symptomatic prior to actual testing. Also complicating this is good evidence that RF exposures testing behavioral changes show delayed results; effects are observed after termination of RF exposure. This suggests a persistent change in the nervous system that may be evident only after time has passed, so is not observed during a short testing period.

The effects of long-term exposure to wireless technologies including emissions from cell phones and other personal devices, and from whole-body exposure to RF transmissions from cell towers and antennas is simply not known yet with certainty. However, the body of evidence at hand suggests that bioeffects and health impacts can and do occur at exquisitely low exposure levels: levels that can be thousands of times below public safety limits.

The evidence reasonably points to the potential for serious public health consequences (and economic costs), which will be of global concern with the widespread public use of, and exposure to such emissions. Even a small increase in disease incidence or functional loss of cognition related to new wireless exposures would have a large public health, societal and economic consequences. Epidemiological studies can report harm to health only after decades of exposure, and where large effects can be seen across "average" populations; so these early warnings of possible harm should be taken seriously now by decision-makers.

C. Effects on Genes (DNA)

Cancer risk is related to DNA damage, which alters the genetic blueprint for growth and development. If DNA is damaged (the genes are damaged) there is a risk that these damaged cells will not die. Instead they will continue to reproduce themselves with damaged DNA, and this is one necessary pre-condition for cancer. Reduced DNA repair may also be an important part of this story. When the rate of damage to DNA exceeds the rate at which DNA can be repaired, there is the possibility of retaining mutations and initiating cancer. Studies on how ELF and RF may affect genes and DNA is important, because of the possible link to cancer.
Even ten years ago, most people believed that very weak ELF and RF fields could not possibly have any effect at all on DNA and how cells work (or are damaged and cannot do their work properly). The argument was that these weak fields are do not possess enough energy (are not physically strong enough) to cause damage. However, there are multiple ways we already know about where energy is not the key factor in causing damage. For example, exposure to toxic chemicals can cause damage. Changing the balance of delicate biological processes, including

hormone balances in the body, can damage or destroy cells, and cause illness. In fact, many chronic diseases are directly related to this kind of damage that does not require any heating at all. Interference with cell communication (how cells interact) may either cause cancer directly or promote existing cancers to grow faster.

Using modern gene-testing techniques will probably give very useful information in the future about how EMFs targets and affects molecules in the body. At the gene level, there is some evidence now that EMFs (both ELF and RF) can cause changes in how DNA works. Laboratory studies have been conducted to see whether (and how) weak EMFs fields can affect how genes and proteins function. Such changes have been seen in some, but not all studies.

Small changes in protein or gene expression might be able to alter cell physiology, and might be able to cause later effects on health and well-being. The study of genes, proteins and EMFs is still in its infancy, however, by having some confirmation at the gene level and protein level that weak EMFs exposures do register changes may be an important step in establishing what risks to health can occur.

What is remarkable about studies on DNA, genes and proteins and EMFs is that there should be no effect at all if it were true that EMFs is too weak to cause damage. Scientists who believe that the energy of EMFs is insignificant and unlikely to cause harm have a hard time explaining these changes, so are inclined to just ignore them. The trouble with this view is that the effects are occurring. Not being able to explain these effects is not a good reason to consider them imaginary or unimportant.

The European research program (REFLEX) documented many changes in normal biological functioning in tests on DNA (3). The significance of these results is that such effects are directly related to the question of whether human health risks might occur, when these changes in genes and DNA happen. This large research effort produced information on EMFs effects from more than a dozen different researchers. Some of the key findings included:

> *"Gene mutations, cell proliferation and apoptosis are caused by or result in altered gene and protein expression profiles. The convergence of these events is required for the development of all chronic diseases."* (3)
>
> *"Genotoxic effects and a modified expression of numerous genes and proteins after EMF exposure could be demonstrated with great certainty.*" (3)
>
> *"RF-EMF produced genotoxic effects in fibroblasts, HL-60 cells, granulosa cells of rats and neural progenitor cells derived from mouse embryonic stem cells."* (Participants 2, 3 and 4). (3)
>
> *"Cells responded to RF exposure between SAR levels of 0.3 and 2 W/Kg with a significant increase in single- and double-strand DNA breaks and in micronuclei frequency."* (Participants 2, 3 and 4). (3)
>
> *"In HL-60 cells an increase in intracellular generation of free radicals accompanying RF-EMF exposure could clearly be demonstrated."* (Participant 2). (3)
>
> *"The induced DNA damage was not based on thermal effects and arouses consideration about the environmental safety limits for ELF-EMF exposure." (3)*

> *"The effects were clearly more pronounced in cells from older donors, which could point to an age-related decrease of DNA repair efficiency of ELF-EMF induced DNA strand breaks."* (3)

Both ELF and RF exposures can be considered genotoxic (will damage DNA) under certain conditions of exposure, including exposure levels that are lower than existing safety limits.

D. Effects on Stress Proteins (Heat Shock Proteins)

In nearly every living organism, there is a special protection launched by cells when they are under attack from environmental toxins or adverse environmental conditions. This is called a stress response, and what are produced are stress proteins (also known as heat shock proteins). Plants, animals and bacteria all produce stress proteins to survive environmental stressors like high temperatures, lack of oxygen, heavy metal poisoning, and oxidative stress (a cause of premature aging). We can now add ELF and RF exposures to this list of environmental stressors that cause a physiological stress response.

Very low-level ELF and RF exposures can cause cells to produce stress proteins, meaning that the cell recognizes ELF and RF exposures as harmful. This is another important way in which scientists have documented that ELF and RF exposures can be harmful, and it happens at levels far below the existing public safety standards.

An additional concern is that if the stress goes on too long, the protective effect is diminished. There is a reduced response if the stress goes on too long, and the protective effect is reduced. This means the cell is less protected against damage, and it is why prolonged or chronic exposures may be quite harmful, even at very low intensities.

The biochemical pathway that is activated is the same for ELF and for RF exposures, and it is non-thermal (does not require heating or induced electrical currents, and thus the safety standards based on protection from heating are irrelevant and not protective). ELF exposure levels of only 5 to 10 mG have been shown to activate the stress response genes (Table 2, Section 6). The specific absorption rate or SAR is not the appropriate measure of biological threshold or dose, and should not be used as the basis for a safety standard, since SAR only regulates against thermal damage.

E. Effects on the Immune System

The immune system is another defense we have against invading organisms (viruses, bacteria, and other foreign molecules). It protects us against illness, infectious diseases, and tumor cells.

There are many different kinds of immune cells; each type of cell has a particular purpose, and is launched to defend the body against different kinds of exposures that the body determines might be harmful.

There is substantial evidence that ELF and RF can cause inflammatory reactions, allergy reactions and change normal immune function at levels allowed by current public safety standards.

The body's immune defense system senses danger from ELF and RF exposures, and targets an immune defense against these fields, much like the body's reaction in producing stress proteins. These are additional indicators that very low intensity ELF and RF exposures are a) recognized by cells and b) can cause reactions as if the exposure is harmful. Chronic exposure to factors that increase allergic and inflammatory responses on a continuing basis are likely to be harmful to health. Chronic inflammatory responses can lead to cellular, tissue and organ damage over time. Many chronic diseases are thought to be related to chronic problems with immune system function.

The release of inflammatory substances, such as histamine, are well-known to cause skin reactions, swelling, allergic hypersensitivity and other conditions that are normally associated with some kind of defense mechanism. The human immune system is part of a general defense barrier that protects against harmful exposures from the surrounding environment. When the immune system is aggravated by some kind of attack, there are many kinds of immune cells that can respond. Anything that triggers an immune response should be carefully evaluated, since chronic stimulation of the immune system may over time impair the system's ability to respond in the normal fashion.

Measurable physiological changes (mast cell increases in the skin, for example that are markers of allergic response and inflammatory cell response) are triggered by ELF and RF at very low intensities. Mast cells, when activated by ELF or RF, will break (degranulate) and release irritating chemicals that cause the symptoms of allergic skin reactions.

There is very clear evidence that exposures to ELF and RF at levels associated with cell phone use, computers, video display terminals, televisions, and other sources can cause these skin reactions. Changes in skin sensitivity have been measured by skin biopsy, and the findings are remarkable. Some of these reactions happen at levels equivalent to those of wireless technologies in daily life. Mast cells are also found in the brain and heart, perhaps targets of immune response by cells responding to ELF and RF exposures, and this might account for some of the other symptoms commonly reported (headache, sensitivity to light, heart arrhythmias and other cardiac symptoms). Chronic provocation by exposure to ELF and RF can lead to immune dysfunction, chronic allergic responses, inflammatory diseases and ill health if they occur on a continuing basis over time.

These clinical findings may account for reports of persons with electrical hypersensitivity, which is a condition where there is intolerance for any level of exposure to ELF and/or RF. Although there is not yet a substantial scientific assessment (under controlled conditions, if that is even possible); anecdotal reports from many countries show that estimates range from 3% to perhaps 5% of populations, and it is a growing problem. Electrical hypersensitivity, like multiple chemical sensitivity, can be disabling and require the affected person to make drastic changes in work and living circumstances, and suffer large economic losses and loss of personal freedom. In Sweden, electrohypersensitivity (EHS) is officially recognized as fully functional impairment (i.e., it is not regarded as a disease – see Section 6, Appendix A).

F. Plausible Biological Mechanisms

Plausible biological mechanisms are already identified that can reasonably account for most biological effects reported for exposure to RF and ELF at low-intensity levels (oxidative stress and DNA damage from free radicals leading to genotoxicity; molecular mechanisms at very low energies are plausible links to disease, e.g., effect on electron transfer rates linked to oxidative damage, DNA activation linked to abnormal biosynthesis and mutation). It is also important to remember that traditional public health and epidemiological determinations do not require a proven mechanism before inferring a causal link between EMFs exposure and disease (12). Many times, proof of mechanism is not known before wise public health responses are implemented.

"Obviously, melatonin's ability to protect DNA from oxidative damage has implications for many types of cancer, including leukemia, considering that DNA damage due to free radicals is believed to be the initial oncostatic event in a majority of human cancers [Cerutti et al., 1994]. In addition to cancer, free radical damage to the central nervous system is a significant component of a variety of neurodegenerative diseases of the aged including Alzheimer's disease and Parkinsonism. In experimental animal models of both of these conditions, melatonin has proven highly effective in forestalling their onset, and reducing their severity [Reiter et al., 2001]." (13)

Oxidative stress through the action of free radical damage to DNA is a plausible biological mechanism for cancer and diseases that involve damage from ELF to the central nervous system.

G. Another Way of Looking at EMFs: Therapeutic Uses

Many people are surprised to learn that certain kinds of EMFs treatments actually can heal. These are medical treatments that use EMFs in specific ways to help in healing bone fractures, to heal wounds to the skin and underlying tissues, to reduce pain and swelling, and for other post-surgical needs. Some forms of EMFs exposure are used to treat depression.

EMFs have been shown to be effective in treating conditions of disease at energy levels far below current public exposure standards. This leads to the obvious question. How can scientists dispute the harmful effects of EMF exposures while at the same time using forms of EMF treatment that are proven to heal the body?

Medical conditions are successfully treated using EMFs at levels below current public safety standards, proving another way that the body recognizes and responds to low-intensity EMF signals. Otherwise, these medical treatments could not work. The FDA has approved EMFs medical treatment devices, so is clearly aware of this paradox.

Random exposures to EMFs, as opposed to EMFs exposures done with clinical oversight, could lead to harm just like the unsupervised use of pharmaceutical drugs. This evidence forms a strong warning that indiscriminate EMF exposure is probably a bad idea.

No one would recommend that drugs used in medical treatments and prevention of disease be randomly given to the public, especially to children. Yet, random and involuntary exposures to EMFs occur all the time in daily life.

The consequence of multiple sources of EMFs exposures in daily life, with no regard to cumulative exposures or to potentially harmful combinations of EMFs exposures means several things. First, it makes it very difficult to do clinical studies because it is almost impossible to find anyone who is not already exposed. Second, people with and without diseases have multiple and overlapping exposures – this will vary from person to person.

Just as ionizing radiation can be used to effectively diagnose disease and treat cancer, it is also a cause of cancer under different exposure conditions. Since EMFs are both a cause of disease, and also used for treatment of disease, it is vitally important that public exposure standards reflect our current understanding of the biological potency of EMF exposures, and develop both new public safety limits and measures to prevent future exposures.

III. EMF EXPOSURE AND PRUDENT PUBLIC HEALTH PLANNING

- **The scientific evidence is sufficient to warrant regulatory action for ELF; and it is substantial enough to warrant preventative actions for RF.**
- **The standard of evidence for judging the emerging scientific evidence necessary to take action should be proportionate to the impacts on health and well-being**
- **The exposures are widespread.**
- **Widely accepted standards for judging the science are used in this assessment.**

Public exposure to electromagnetic radiation (power-line frequencies, radiofrequency and microwave) is growing exponentially worldwide. There is a rapid increase in electrification in developing countries, even in rural areas. Most members of society now have and use cordless phones, cellular phones, and pagers. In addition, most populations are also exposed to antennas in communities designed to transmit wireless RF signals. Some developing countries have even given up running land lines because of expense and the easy access to cell phones. Long-term and cumulative exposure to such massively increased RF has no precedent in human history. Furthermore, the most pronounced change is for children, who now routinely spend hours each day on the cell phone. Everyone is exposed to a greater or lesser extent. No one can avoid exposure, since even if they live on a mountain-top without electricity there will likely be exposure to communication-frequency RF exposure. Vulnerable populations (pregnant women, very young children, elderly persons, the poor) are exposed to the same degree as the general population. Therefore it is imperative to consider ways in which to evaluate risk and reduce exposure. Good public health policy requires preventative action proportionate to the potential risk of harm and the public health consequence of taking no action.

IV. RECOMMENDED ACTIONS

A. Defining new exposure standards for ELF

This chapter concludes that new ELF limits are warranted based on a public health analysis of the overall existing scientific evidence. The public health view is that new ELF limits are needed now. They should reflect environmental levels of ELF that have been demonstrated to increase risk for childhood leukemia, and possibly other cancers and neurological diseases. ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor. It is no longer acceptable to build new power lines and electrical facilities that place people in ELF environments that have been determined to be risky. These levels are in the 2 to 4 milligauss* (mG) range, not in the 10s of mG or 100s of mG. The existing ICNIRP limit is 1000 mG (904 mG in the US) for ELF is outdated and based on faulty assumptions. These limits are can no longer be said to be protective of public health and they should be replaced. A safety buffer or safety factor should also be applied to a new, biologically-based ELF limit, and the conventional approach is to add a safety factor lower than the risk level.

While new ELF limits are being developed and implemented, a reasonable approach would be a 1 mG planning limit for habitable space adjacent to all new or upgraded power lines and a 2 mG limit for all other new construction. It is also recommended for that a 1 mG limit be established for existing habitable space for children and/or women who are pregnant (because of the possible link between childhood leukemia and *in utero* exposure to ELF). This recommendation is based on the assumption that a higher burden of protection is required for children who cannot protect themselves, and who are at risk for childhood leukemia at rates that are traditionally high enough to trigger regulatory action. This situation in particular warrants extending the 1 mG limit to existing occupied space. "Establish" in this case probably means formal public advisories from relevant health agencies. While it is not realistic to reconstruct all existing electrical distribution systems, in the short term; steps to reduce exposure from these existing systems need to be initiated, especially in places where children spend time, and should be encouraged. These limits should reflect the exposures that are commonly associated with increased risk of child hood leukemia (in the 2 to 5 mG range for all children, and over 1.4 mG for children age 6 and younger). Nearly all of the occupational studies for adult cancers and neurological diseases report their highest exposure category is 4 mG and above, so that new ELF limits should target the exposure ranges of interest, and not necessarily higher ranges.

Avoiding chronic ELF exposure in schools, homes and the workplace above levels associated with increased risk of disease will also avoid most of the possible bioactive parameters of ELF discussed in the relevant literature.

B. Defining preventative actions for reduction in RF exposures

Given the scientific evidence at hand (Chapter 17), the rapid deployment of new wireless technologies that chronically expose people to pulsed RF at levels reported to cause bioeffects, which in turn, could reasonably be presumed to lead to serious health impacts, is of public health concern. Section 17 summarizes evidence that has resulted in a public health recommendation that preventative action is warranted to reduce or minimize RF exposures to the public. There is suggestive to strongly suggestive evidence that RF exposures may cause changes in cell membrane function, cell communication, cell metabolism, activation of proto-oncogenes and can trigger the production of stress proteins at exposure levels below current regulatory limits. Resulting effects can include DNA breaks and chromosome aberrations, cell death including death of brain neurons, increased free radical production, activation of the endogenous opioid system, cell stress and premature aging, changes in brain function including memory loss, retarded learning, slower motor function and other performance impairment in children, headaches and fatigue, sleep disorders, neurodegenerative conditions, reduction in melatonin secretion and cancers (Chapters 5, 6, 7, 8, 9, 10, and 12).

As early as 2000, some experts in bioelectromagnetics promoted a 0.1 µW/cm2 limit (which is 0.614 Volts per meter) for ambient outdoor exposure to pulsed RF, so generally in cities, the public would have adequate protection against involuntary exposure to pulsed radiofrequency (e.g., from cell towers, and other wireless technologies). The Salzburg Resolution of 2000 set a target of 0.1 µW/cm2 (or 0.614 V/m) for public exposure to pulsed radiofrequency. Since then, there are many credible anecdotal reports of unwellness and illness in the vicinity of wireless transmitters (wireless voice and data communication antennas) at lower levels. Effects include sleep disruption, impairment of memory and concentration, fatigue, headache, skin disorders, visual symptoms (floaters), nausea, loss of appetite, tinnitus, and cardiac problems (racing heartbeat), There are some credible articles from researchers reporting that cell tower -level RF exposures (estimated to be between 0.01 and 0.5 µW/cm2) produce ill-effects in populations living up to several hundred meters from wireless antenna sites.

This information now argues for thresholds or guidelines that are substantially below current FCC and ICNIPR standards for whole body exposure. Uncertainty about how low such standards might have to go to be prudent from a public health standpoint should not prevent reasonable efforts to respond to the information at hand. No lower limit for bioeffects and adverse health effects from RF has been established, so the possible health risks of wireless WLAN and WI-FI systems, for example, will require further research and no assertion of safety at any level of wireless exposure (chronic exposure) can be made at this time. The lower limit for reported human health effects has dropped 100-fold below the safety standard (for mobile phones and PDAs); 1000- to 10,000-fold for other wireless (cell towers at distance; WI-FI and WLAN devices). The entire basis for safety standards is called into question, and it is not unreasonable to question the safety of RF at any level.

A cautionary target level for pulsed RF exposures for ambient wireless that could be applied to RF sources from cell tower antennas, WI-FI, WI-MAX and other similar sources is proposed. The recommended cautionary target level is 0.1 microwatts per centimeter squared (µW/cm2)** (or 0.614 Volts per meter or V/m)** for pulsed RF where these exposures affect the general public; this advisory is proportionate to the evidence and in accord with prudent public health policy. A precautionary limit of 0.1 µW/cm2 should be adopted for outdoor, cumulative RF exposure. This reflects the current RF science and prudent public health response that would reasonably be set for pulsed RF (ambient) exposures where people live, work and go to school. This level of RF is experienced as whole-body exposure, and can be a chronic exposure where there is wireless coverage present for voice and data transmission for cell phones, pagers and PDAs and other sources of radiofrequency radiation. An outdoor precautionary limit of 0.1 µW/cm2 would mean an even lower exposure level inside buildings, perhaps as low as 0.01 µW/cm2. Some studies and many anecdotal reports on ill health have been reported at lower levels than this; however, for the present time, it could prevent some of the most disproportionate burdens placed on the public nearest to such installations. Although this RF target level does not preclude further rollout of WI-FI technologies, we also recommend that wired alternatives to WI-FI be implemented, particularly in schools and libraries so that children are not subjected to elevated RF levels until more is understood about possible health impacts. This recommendation should be seen as an interim precautionary limit that is intended to guide preventative actions; and more conservative limits may be needed in the future.

Broadcast facilities that chronically expose nearby residents to elevated RF levels from AM, FM and television antenna transmission are also of public health concern given the potential for very high RF exposures near these facilities (antenna farms). RF levels can be in the 10s to several 100's of µW/cm2 in residential areas within half a mile of some broadcast sites (for example, Lookout Mountain, Colorado and Awbrey Butte, Bend, Oregon). Such facilities that are located in, or expose residential populations and schools to elevated levels of RF will very likely need to be re-evaluated for safety.

For emissions from wireless devices (cell phones, personal digital assistant or PDA devices, etc) there is enough evidence for increased risk of brain tumors and acoustic neuromas now to warrant intervention with respect to their use. Redesign of cell phones and PDAs could prevent direct head and eye exposure, for example, by designing new units so that they work only with a wired headset or on speakerphone mode.

These effects can reasonably be presumed to result in adverse health effects and disease with chronic and uncontrolled exposures, and children may be particularly vulnerable. The young are also largely unable to remove themselves from such environments. Second-hand radiation, like second-hand smoke is an issue of public health concern based on the evidence at hand.

V. CONCLUSIONS

- We cannot afford ‘business as usual” any longer. It is time that planning for new power lines and for new homes, schools and other habitable spaces around them is done with routine provision for low-ELF environments. The business-as-usual deployment of new wireless technologies is likely to be risky and harder to change if society does not make some educated decisions about limits soon. Research must continue to define what levels of RF related to new wireless technologies are acceptable; but more research should not prevent or delay substantive changes today that might save money, lives and societal disruption tomorrow.

- New regulatory limits for ELF are warranted. ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor. It is no longer acceptable to build new power lines and electrical facilities that place people in ELF environments that have been determined to be risky (at levels generally at 2 mG and above).

- While new ELF limits are being developed and implemented, a reasonable approach would be a 1 mG planning limit for habitable space adjacent to all new or upgraded power lines and a 2 mG limit for all other new construction, It is also recommended for that a 1 mG limit be established for existing habitable space for children and/or women who are pregnant . This recommendation is based on the assumption that a higher burden of protection is required for children who cannot protect themselves, and who are at risk for childhood leukemia at rates that are traditionally high enough to trigger regulatory action. This situation in particular warrants extending the 1 mG limit to existing occupied space. "Establish" in this case probably means formal public advisories from relevant health agencies.

- While it is not realistic to reconstruct all existing electrical distributions systems, in the short term; steps to reduce exposure from these existing systems need to be initiated, especially in places where children spend time, and should be encouraged.

- A precautionary limit of 0.1 (µW/cm2 (which is also 0.614 Volts per meter) should be adopted for outdoor, cumulative RF exposure. This reflects the current RF science and prudent public health response that would reasonably be set for pulsed RF (ambient) exposures where people live, work and go to school. This level of RF is experienced as whole-body exposure, and can be a chronic exposure where there is wireless coverage present for voice and data transmission for cell phones, pagers and PDAs and other sources of radiofrequency radiation. Some studies and many anecdotal reports on ill health have been reported at lower levels than this; however, for the present time, it could prevent some of the most disproportionate burdens placed on the public nearest to such installations. Although this RF target level does not preclude further rollout of WI-FI technologies, we also recommend that wired alternatives to WI-FI be implemented, particularly in schools and libraries so that children are not subjected to elevated RF levels until more is understood about possible health impacts. This recommendation should be seen as an interim precautionary limit that is intended to guide preventative actions; and more conservative limits may be needed in the future.

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Some Quick Definitions for Units of Measurement of ELF and RF

***Milligauss (mG)**

A milligauss is a measure of ELF intensity and is abbreviated mG. This is used to describe electromagnetic fields from appliances, power lines, interior electrical wiring.

****Microwatts per centimeter squared (µW/cm2)**

Radiofrequency radiation in terms of power density is measured in microwatts per centimeter squared and abbreviated (µW/cm2). It is used when talking about emissions from wireless facilities, and when describing ambient RF in the environment. The amount of allowable RF near a cell tower is 1000 µW/cm2 for some cell phone frequencies, for example.

*****Specific Absorption Rate (SAR is measured in watts per kilogram or W/Kg)**

SAR stands for specific absorption rate. It is a calculation of how much RF energy is absorbed into the body, for example when a cell phone or cordless phone is pressed to the head. SAR is expressed in watts per kilogram of tissue (W/Kg). The amount of allowable energy into 1 gram of brain tissue from a cell phone is 1.6 W/Kg in the US. For whole body exposure, the exposure is 0.8 W/Kg averaged over 30 minutes for the general public. International standards in most countries are similar, but not exactly the same.

Table 1-1 BioInitiative Report Overall Conclusions

OVERALL SUMMARY OF CONCLUSIONS

- The existing ICNIRP and FCC limits for public and occupational exposure to ELF and RF are insufficiently protective of public health.

- Biologically-based public and occupational exposure standards for extra-low frequency and radiofrequency radiation are recommended to address bioeffects and potential adverse health effects of chronic exposure to ELF and RF. These effects are now widely reported to occur at exposure levels significantly below most current national and international limits.

- A biologically-based exposure limit is one that is protective against ELF and RF intensity and modulation factors which, with chronic exposure, can reasonably be presumed to result in significant impacts to health and well-being.

- Research is needed (but should not delay) regulatory action for ELF and substantive *preventative action* for RF proportionate to potential health and wellbeing risks from chronic exposure.

- A biologically-based exposure limit should reflect current scientific knowledge of bioeffects and health effects, and impose new limits based on preventative action as defined by the Precautionary Principle (EEA, 2001).

- Biologically-based exposure standards shall be protective against exposures levels of ELF and RF that affect or change normal biological functioning of organisms (humans). They shall not be based solely on energy absorption or thermal levels of energy input, or resulting tissue heating. They shall be protective against chronic exposure responses.

- The existing standards are based on thermal (heating) limits, and do not address non-thermal (or low-intensity) exposures which are widely reported to cause bioeffects, some likely leading to adverse health effects with chronic exposure.

- Biological effects may include both potential adverse health effects and loss of homeostasis and well-being.

- Biologically-based exposure standards are needed to prevent disruption of normal body processes. Effects are reported for DNS damage (genotoxicity that is directly linked to integrity of the human genome), cellular communication, cellular metabolism and repair, cancer surveillance within the body; and for protection against cancer and neurological diseases. Also reported are neurological effects including impairment of sleep and sleep architecture, cognitive function and memory; depression; cardiac effects; pathological leakage of the blood-brain barrier; and impairment of normal immune function, fertility and reproduction.

- Frequency, intensity, exposure duration, and the number of exposure episodes can affect the response, and these factors can interact with each other to produce different effects. In addition, in order to understand the biological consequences of EMF exposure, one must know whether the effect is cumulative, whether compensatory responses result, and when homeostasis will break down.

- Plausible biological mechanisms that can account for genotoxicity (DNA damage) are already well known (oxidative damage via free-radical actions) although it should also be said that there is not yet proof. *However, proof of mechanism is not required to set prudent public health policy, nor is it mandatory to set new guidelines or limits if adverse health effects occur at lower-than-existing IEEE and ICNIRP standards.*

Table 1-1 BioInitiative Report Overall Conclusions

OVERALL SUMMARY OF CONCLUSIONS (continued

- The SCENIHR report (2007) states that “for breast cancer and cardiovascular disease, recent research has indicated that an association with EMF is unlikely.” The WHO ELF Health Criteria Monograph (2007) states “The evidence does not support an association between ELF exposure and cardiovascular disease” and “(T)he evidence for breast cancer was also considered to be effectively negative, while for other diseases it was judged to be inadequate.” Neither conclusion is supported by any finding by IARC that would classify EMF as Class 4 (Not A Carcinogen), so it is premature for either group to dismiss the evidence for EMF as a potential risk factor for either breast cancer or for cardiovascular disease.

- The standard for taking action should be precautionary; action should not be deferred while waiting for final proof or causal evidence to be established that EMF is harmful to health and well-being.

- There is great public concern over increasing levels of involuntary exposure to radiofrequency and ELF-modulated radiofrequency exposures from new wireless technologies; there is widespread public resistance to radiofrequency and extra-low frequency radiation exposures which are allowable under current, thermally-based exposure standards.

- There is inadequate warning and notice to the public about possible risks from wireless technologies in the marketplace, which is resulting in adoption and use of technologies that may have adverse health consequences which are still unknown to the public. There is no “informed consent”.

- No positive assertion of safety can be made by governments that continue to support and enforce exposure limits for RF and ELF based on ICNIRP or IEEE criteria (or the equivalent). Governments that are considering proposals to relax existing RF and ELF standards should reject these proposals given the weight of scientific evidence that is available; and the clear disconnect between existing public safety limits and their responsibility to provide safe and healthful living environments for all segments of affected populations.

Section 5 Genotoxicity Based on Proteomics

- EMF exposure can change gene and/or protein expression in certain types of cells, even at intensities lower than ICNIRP recommended values.
- The biological consequences of most of the changed genes/proteins are still unclear, and need to be further explored.
- The EMF research community should pay equal attention to the negative reports as to the positive ones. Not only the positive findings need to be replicated, all the negative ones are also needed to be validated.
- The IEEE and WHO data bases do not include the majority of ELF studies (only 6 of 14 in the WHO; 0 of 16 in IEEE); they do include the majority of the RF studies (14 of 16).

Table 1-1 BioInitiative Report Overall Conclusions

Section 6 Genotoxicity (DNA Damage from RF and ELF)

- Toxicity to the genome can lead to a change in cellular functions, cancer, and cell death. One can conclude that under certain conditions of exposure RF is genotoxic. Data available are mainly applicable only to cell phone radiation exposure. One study reports that RF at levels equivalent to the vicinity of base stations and RF- transmission towers is genotoxic and could cause DNA damage (Phillips et al., 1998).

- RF may be considered genotoxic (cause DNA damage). Of 28 total studies on radiofrequency radiation (RF) and DNA damage, 14 studies reported effects (50%) and 14 reported no significant effect (50%). Of 29 total studies on radiofrequency radiation and micronucleation, 16 studies reported effects (55%) and 13 reported no significant effect (45%). Of 21 total studies on chromosome and genome damage from radiofrequency radiation, 13 studies (62%) reported effects and 8 studies (38%) reported no significant effects.

- During cell phone use, a relatively constant mass of tissue in the brain is exposed to radiation at relatively high intensity (peak SAR of 4 - 8 W/kg). Several studies have reported DNA damage at lower than 4 W/kg.

- Since critical genetic mutations in one single cell are sufficient to lead to cancer and there are millions of cells in a gram of tissue, *it is* ***inconceivable*** that the base of the IEEE SAR standard was changed from averaged over 1 gram of tissue to 10 grams.

- Frequency, intensity, exposure duration, and the number of exposure episodes can affect the response, and these factors can interact with each other to produce different consequences. In order to understand the biological consequence of exposure, one must understand whether the effect is cumulative, whether compensatory responses result and when homeostasis will break down. The choice of cell type or organism studied can also influence the outcome.

- Extremely-low frequency (ELF) has also been shown to be genotoxic and cause DNA damage. Of 41 relevant studies of genotoxicity and ELF exposure, 27 studies (66%) report DNA damage and 14 studies (44%) report no significant effect.

Table 1-1 BioInitiative Report Overall Conclusions

Section 7: Stress Response

- Scientific research on stress proteins has shown that the public is not being protected from potential damage that can be caused by exposure to EMF, both power frequency (ELF) and radio frequency (RF).

- Cells react to an EMF as potentially harmful by producing stress proteins (heat shock proteins or hsp).

- Direct interaction of ELF and RF with DNA has been documented and both activate the synthesis of stress proteins.

- The biochemical pathway that is activated is the same pathway in both ELF and RF and it is non-thermal.

- Many biological systems are affected by EMFs (meaning both ELF and RF trigger stress proteins).

- Many frequencies are active. Field strength and exposure duration thresholds are very low.

- Molecular mechanisms at very low energies are plausible links to disease (e.g., effect on electron transfer rates linked to oxidative damage, DNA activation linked to abnormal biosynthesis and mutation). Cells react to an EMF as potentially harmful.

- Many lines of research now point to changes in DNA electron transfer as a plausible mechanism of action as a result of non-thermal ELF and RF.

- The same biological reaction (production of stress proteins) to an EMF can be activated in more than one division of the EM spectrum.

- Direct interaction of ELF and RF with DNA has been documented and both activate the synthesis of stress proteins.

- Thresholds triggering stress on biological systems occur at environment levels on the order of 0.5 to 1.0 µT for ELF.

- DNA damage (e.g., strand breaks), a cause of cancer, occurs at levels of ELF and RF that are below the safety limits. Also, there is no protection against cumulative effects stimulated by different parts of the EM spectrum.

- The scientific basis for EMF safety limits is flawed when the same biological mechanisms are activated in ELF and RF ranges at vastly different levels of the Specific Absorption Rate (SAR). Activation of DNA to synthesize stress proteins (the stress response) is stimulated in the ELF at a non-thermal SAR level that is over a billion times lower than the same process activated by RF at the thermal level.

- There is a need for a biological standard to replace the thermal standard and to also protect against cumulative effects across the EM spectrum.

- Based on studies of stress proteins, the specific absorption rate (SAR) is not the appropriate measure of biological threshold or dose, and should not be used as a basis for a safety standard since it regulates against thermal effects only.

Table 1-1 BioInitiative Report Overall Conclusions

Section 8 Effects on Immune Function

- Both human and animal studies report large immunological changes with exposure to environmental levels of electromagnetic fields (EMFs). Some of these exposure levels are equivalent to those of e.g. wireless technologies in daily life.

- Measurable physiological changes (mast cells increases, for example) that are bedrock indicators of allergic response and inflammatory conditions are stimulated by EMF exposures.

- Chronic exposure to such factors that increase allergic and inflammatory responses on a continuing basis may be harmful to health.

- It is possible that chronic provocation by exposure to EMF can lead to immune dysfunction, chronic allergic responses, inflammatory responses and ill health if they occur on a continuing basis over time. This is an important area for future research.

- Specific findings from studies on exposures to various types of modern equipment and/or EMFs report over-reaction of the immune system; morphological alterations of immune cells; profound increases in mast cells in the upper skin layers, increased degranulation of mast cells and larger size of mast cells in electrohypersensitive individuals; presence of biological markers for inflammation that are sensitive to EMF exposure at non-thermal levels; changes in lymphocyte viability; decreased count of NK cells; decreased count of T lymphocytes; negative effects on pregnancy (uteroplacental circulatory disturbances and placental dysfunction with possible risks to pregnancy); suppressed or impaired immune function; and inflammatory responses which can ultimately result in cellular, tissue and organ damage.

- Electrical hypersensitivity is reported by individuals in the United States, Sweden, Switzerland, Germany. Denmark and many other countries of the world. Estimates range from 3% to perhaps 10% of populations, and appears to be a growing condition of ill-health leading to lost work and productivity.

- The WHO and IEEE literature surveys do not include all of the relevant papers cited here, leading to the conclusion that evidence has been ignored in the current WHO ELF Health Criteria Monograph; and the proposed new IEEE C95.1 RF public exposure limits (April 2006).

- The current international public safety limits for EMFs do not appear to be sufficiently protective of public health at all, based on the studies of immune function. New, biologically-based public standards are warranted that take into account low-intensity effects on immune function and health that are reported in the scientific literature.

Table 1-1 BioInitiative Report Overall Conclusions

Section 9 Neurology and Behavioral Effects

- Effects on neurophysiological and cognitive functions are quite well established.

- Studies on EEG and brain evoked-potentials in humans exposed to cellular phone radiation predominantly showed positive effects (i.e., positive means the exposure has the ability to change brainwave activity even at exposure levels where no effect would be expected, based on traditional understanding and safety limits).

- There is little doubt that electromagnetic fields emitted by cell phones and cell phone use affect electrical activity in the brain.

- The behavioral consequences of these neuroelectrophysiological changes are not always predictable and research on electrophysiology also indicates that effects are dependent on the mental load of the subjects during exposure, e.g., on the complexity of the task that a subject is carrying out.

- Most of the studies carried out so far are short-term exposure experiments, whereas cell phone use causes long-term repeated exposure of the brain.

- In most of the behavioral experiments, effects were observed after the termination of RF exposure. In some experiments, tests were made days after exposure. This suggests a persistent change in the nervous system after exposure to RF.

- In many instances, neurological and behavioral effects were observed at a SAR less than 4 W/kg. This directly contradicts the basic assumption of the IEEE guideline criterion.

- Caution should be taken in concluding that a neurological effect resulted solely from the action of RF on the central nervous system because it is well known that the functions of the central nervous system can be affected by activity in the peripheral nervous system.

Table 1-1 BioInitiative Report Overall Conclusions

Section 10 Brain Tumors and Acoustic Neuromas

- Studies on brain tumors and use of mobile phones for ≥ 10 years gave a consistent pattern of an increased risk for acoustic neuroma and glioma.

- Cell phone use > 10 years give a consistent pattern of an increased risk for acoustic neuroma and glioma, most pronounced for high-grade glioma. The risk is highest for ipsilateral exposure.

Section 10 Brain Tumors and RF - Epidemiology

- Only a few studies of long-term exposure to low levels of RF fields and brain tumors exist, all of which have methodological shortcomings including lack of quantitative exposure assessment. Given the crude exposure categories and the likelihood of a bias towards the null hypothesis of no association, *the body of evidence is consistent with a moderately elevated risk.*

- Occupational studies indicate that long-term exposure at workplaces may be associated with an elevated brain tumor risk.

- Although the population attributable risk is low (likely below 4%), still more than 1,000 cases per year in the US can be attributed to RF exposure at workplaces alone. Due to the lack of conclusive studies of environmental RF exposure and brain tumors the potential of these exposures to increase the risk cannot be estimated.

- Overall, the evidence suggests that long-term exposure to levels generally below current guideline levels still carry the risk of increasing the incidence of brain tumors.

- Epidemiological studies as reviewed in the IEEE C95.1 revision (2006) are deficient to the extent that the entire analysis is professionally unsupportable. IEEEs dismissal of epidemiological studies that link RF exposure to cancer endpoints should be disregarded, as well as any IEEE conclusions drawn from this flawed analysis of epidemiological studies.

Table 1-1 BioInitiative Report Overall Conclusions

Brain Tumors and Acoustic Neuromas

Additional Data from Section 10

- Mobile phone use increases the risk of acoustic neuroma for persons using a mobile phone 10 years or longer by 30% (when used on both sides of head) to 240% (habitually used on one side of head). This information relies on a meta-analysis of several major studies. For acoustic neuroma studies by Lönn et al., (2004), Christensen et al., (2004) Schoemaker et al., (2005) and Hardell et al., (2006a) all giving results for at least 10 years latency period or more. Overall OR = 1.3, 95 % CI = 0.6-2.8 was obtained increasing to OR = 2.4, 95 % CI = 1.1-5.3 for ipsilateral mobile phone use (Lönn et al., 2004, Schoemaker et al., 2005, Hardell et al., 2006).

- There is observational support for the association between acoustic neuroma and the use of mobile phones since some studies report that the tumor is often located in an anatomical area with high exposure during calls with cellular or cordless phones (Hardell et al., 2003).

- Mobile phone use increases the risk of brain tumors (glioma) for persons using a mobile phone 10 years or longer by 20% (when used on both sides of head) to 200% (habitually used on one side of head). This information relies on a meta-analysis of several major studies. For glioma OR = 1.2, [95 % CI = 0.8-1.9] was calculated (Lönn et al., 2005, Christensen et al., 2005, Hepworth et al., 2006, Schüz et al., 2006, Hardell et al., 2006b, Lahkola et al., 2007). Ipsilateral use yielded OR = 2.0, [95 % CI = 1.2-3.4](Lönn et al., 2005, Hepworth et al., 2006, Hardell et al., 2006b, Lahkola et al., 2007).

- Cordless phone use is also associated with an increased risk for acoustic neuromas and brain tumors (both low-and high-grade gliomas (Hardell et al., 2006 a,b).

- The increased risk of acoustic neuroma from use of a cordless phone for ten years or more was reported to be 310% higher risk (when the cordless phone habitually used on the same-side of the head) in Hardell et al., 2006a.

- The increased risk of high-grade glioma from use of a cordless phone for ten years or more was reported to be 220% higher risk (when cordless used on both sides of head) to 470% higher risk (when cordless used habitually on same side of head) in Hardell et al., 2006b.

- The increased risk of low-grade glioma from use of a cordless phone for ten years or more was reported to be 60% higher risk (when cordless used on both sides of head) to 320% higher risk (when cordless used habitually on same side of head) in Hardell et al., 2006b.

- The current standard for exposure to microwaves during mobile phone use and for cordless phone use is not safe considering studies reporting long-term brain tumor risk.

Table 1-1 BioInitiative Report Overall Conclusions

Section 11 Leukemia

- The balance of evidence suggests that childhood leukemia is associated with exposure to power frequency EMFs either during early life or pregnancy.

- Considering only average ELF (MF flux densities) the population attributable risk is low to moderate. However there is a possibility that other exposure metrics are much more strongly related to childhood leukemia and may account for a substantial proportion of cases. The population attributable fraction ranges between 1-4% (Kheifets et al., 2007); 2-4% (Greenland & Kheifets 2006); and 3.3% (Greenland, 2001) assuming only exposures above 3 to 4 mG (0.3 – 0.4 µT) are relevant. However, if it is not average ELF (average MF flux density) that is the metric causally related to childhood leukemia the attributable fraction can be much higher. Up to 80% of childhood leukemia may be caused by exposure to ELF.

- Other childhood cancers except leukemia have not been studied in sufficient detail to allow conclusions about the existence and magnitude of the risk.

- IEEE guideline levels are designed to protect from short-term immediate effects, long-term effects, such as cancer are evoked by levels several orders of magnitudes below current guideline levels.

- Measures should be implemented to guarantee that exposure due to transmission and distribution lines is below an average of about 1 mG (0.1 µT) and precautionary measures are warranted that can reduce all aspects of exposure.

Table 1-1 BioInitiative Report Overall Conclusions

Section 12 Melatonin, Alzheimers Disease and Breast Cancer

- There is strong epidemiologic evidence that long-term exposure to ELF magnetic field (MF) is a risk factor for Alzheimers disease.

- There is now evidence that 1) high levels of peripheral amyloid beta are a risk factor for AD and 2) medium to high MF exposure can increase peripheral amyloid beta. High brain levels of amyloid beta are also a risk factor for AD and medium to high MF exposure to brain cells likely also increases these cells' production of amyloid beta.

- There is considerable *in vitro* and animal evidence that melatonin protects against Alzheimer's disease. Therefore it is certainly possible that low levels of melatonin production are associated with an increase in the risk of AD.

- There are insufficient studies to formulate an opinion as to whether radiofrequency MF exposure is a risk factor for AD.

- Some studies on EMF show reduced melatonin levels, There is sufficient evidence from *in vitro* and animal studies, from human biomarker studies, from occupational and light-at-night studies, and a single longitudinal study with appropriate collection of urine samples to conclude that high MF exposure may be a risk factor for breast cancer.

- There is rather strong evidence from case-control studies that longterm, high occupational exposure (≥ 10 mG or 1.0 µT)) to ELF magnetic fields is a risk factor for breast cancer.

- Seamstresses are, in fact, one of the most highly MF exposed occupations, with exposure levels generally above 10 mG (1.0 µT) over a significant proportion of the workday. They have also been consistently found to be at higher risk of Alzheimer's disease and (female) breast cancer. This occupation deserves attention in future studies.

- There are no studies of RF magnetic fields on breast cancer that do not exclude ELF magnetic field, so that predictions of RF magnetic field alone on breast cancer cannot be assessed at this time.

Table 1-1 BioInitiative Report Overall Conclusions

Section 13 Melatonin – Cell and Animal Studies

- An association between power-frequency electromagnetic fields (ELF) and breast cancer is strongly supported in the scientific literature by a constellation of relevant scientific papers providing mutually-reinforcing evidence from cell and animal studies.

- ELF at environmental levels negatively affects the oncostatic effects of both melatonin and tamoxifen on human breast cancer cells at common environmental levels of ELF exposure at 6 to 12 mG (0.6 to 1.2 µT). Epidemiological studies over the last two decades have reported increased risk of male and female breast cancer with exposures to residential and occupational levels of ELF. Animal studies have reported increased mammary tumor size and incidence in association with ELF exposure.

- ELF limits for public exposure should be revised to reflect increased risk of breast cancer at environmental levels possibly as low as 2 mG or 3 mG (o.2 to 0.3 µT); certainly as low as 4 mG (0.4 µT).

Section 14 Effects of Modulation of Signal

- There is substantial scientific evidence that some modulated fields (pulsed or repeated signals) are bioactive, which increases the likelihood that they could have health impacts with chronic exposure even at very low exposure levels.

- Modulation signals may interfere with normal, non-linear biological processes.

- Modulation is a fundamental factor that should be taken into account in new public safety standards; at present it is not even a contributing factor.

- To properly evaluate the biological and health impacts of exposure to modulated RF (carrier waves), it is also essential to study the impact of the modulating signal (lower frequency fields or ELF-modulated RF).

.• Current standards have ignored modulation as a factor in human health impacts, and thus are inadequate in the protection of the public in terms of chronic exposure to some forms of ELF-modulated RF signals.

- The current IEEE and ICNIRP standards are not sufficiently protective of public health with respect to chronic exposure to modulated fields (particularly new technologies that are pulse-modulated and heavily used in cellular telephony).

Table 1-1 BioInitiative Report Overall Conclusions

Section 14 Effects of Modulation of Signal (continued)

- The collective papers on modulation appear to be omitted from consideration in the recent WHO and IEEE science reviews. This body of research has been ignored by current standard setting bodies that rely only on traditional energy-based (thermal) concepts.

- More research is needed to determine which modulation factors, and combinations are bioactive and deleterious at low intensities, and are likely to result in disease-related processes and/or health risks; however this should not delay preventative actions supporting public health and wellness.

- If signals need to be modulated in the development of new wireless technologies, for example, it makes sense to use what existing scientific information is available to avoid the most obviously deleterious exposure parameters and select others that may be less likely to interfere with normal biological processes in life.

- The current membership on Risk Assessment committees needs to be made more inclusive, by adding scientists experienced with the research reporting non-thermal biological effects.

- The current practice of segregating scientific investigations (and resulting public health limits) by artificial divisions of frequency needs to be changed because this approach dramatically dilutes the impact of the basic science results and eliminates consideration of modulation signals, thereby reducing and distorting the weight of evidence in any evaluation process.

Section 15 Therapeutic Uses of EMF at Low-Intensity Levels

- EMFs are both a cause of disease, and also used for treatment of disease (at levels far below existing public exposure standards).

- Electromagnetic fields are widely used in therapeutic medical applications.

- Proof of effectiveness has been demonstrated in numerous clinical applications of low-intensity ELF and RF.

- EMFs have been shown to be effective in treating conditions of disease at energy levels far below current public exposure standards.

- Indiscriminate EMF exposure is ill advised at even at common environmental levels.

- Multiple sources of EMF exposure in daily life, and cumulative exposures to potentially harmful combinations of EMF are ignored – we don’t even study it properly yet.

Table 1-1 BioInitiative Report Overall Conclusions

Section 16 The Precautionary Principle

- The Precautionary Principle has been developed to help justify public policy action on the protection of health where there are plausible, serious and irreversible hazards from current and future exposures and where there are many uncertainties and much scientific ignorance. EMF is characterized by such circumstances.

- The lessons from the histories of most well known hazards show that precautionary- based yet proportionate measures taken in response to robust early warnings can avoid the kinds of costs incurred by asbestos, smoking, PCBs ,X rays etc. Such lessons are relevant to the EMF issue.

- Policymakers need to be aware of the systematic biases within the environmental health science against finding a true hazard, in order to not compromise scientific integrity. However, this bias can lead to the health of people or environments being compromised.

- The Precautionary Principle introduces the use of different levels of proof (or strengths of evidence) to justify actions to reduce exposure, where the level of proof chosen depends upon the nature and distribution of the costs of being wrong in acting, or not acting; the benefits of the agent or substance in question; the availability of alternatives, etc. Waiting for high levels of scientific proof of causality, or for knowledge about mechanisms of action, can be very expensive in terms of compensation, health care, job losses, reductions in public trust of scientists etc.

- The level of proof chosen to justify action does not determine any particular policy measure, or type of action. This is dependent on factors such as the costs of different measures, equity, the origins of the risk, ie voluntary or imposed, etc.

- There is a need to involve stakeholders in helping to frame problems for risk assessments and to choose appropriate levels of proof and types of actions to reduce exposure.

Table 1-1 BioInitiative Report Overall Conclusions

Section 17: Key Scientific Evidence and Public Health Policy Recommendations

- We cannot afford 'business as usual" any longer. It is time that planning for new power lines and for new homes, schools and other habitable spaces around them is done with provision for low-ELF environments. The business-as-usual deployment of new wireless technologies is likely to be risky and harder to change if society does not make some educated decisions about limits soon. Research must continue to define what levels of RF related to new wireless technologies are acceptable; but more research should not prevent or delay substantive changes today that might save money, lives and societal disruption tomorrow.

- New regulatory limits for ELF are warranted. ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor. It is no longer acceptable to build new power lines and electrical facilities that place people in ELF environments that have been determined to be risky (at levels generally at 2 mG (0.2 μT) and above).

- While new ELF limits are being developed and implemented, a reasonable approach would be a 1 mG (0.1 μT) planning limit for habitable space adjacent to all new or upgraded power lines and a 2 mG (0.2 μT) limit for all other new construction, It is also recommended for that a 1 mG (0,1 μT) limit be established for existing habitable space for children and/or women who are pregnant . This recommendation is based on the assumption that a higher burden of protection is required for children who cannot protect themselves, and who are at risk for childhood leukemia at rates that are traditionally high enough to trigger regulatory action. This situation in particular warrants extending the 1 mG (0.1 μT) limit to existing occupied space. "Establish" in this case probably means formal public advisories from relevant health agencies.

- While it is not realistic to reconstruct all existing electrical distributions systems, in the short term; steps to reduce exposure from these existing systems need to be initiated, especially in places where children spend time, and should be encouraged.

- A precautionary limit of 0.1 μW/cm2 (which is also 0.614 Volts per meter) should be adopted for outdoor, cumulative RF exposure. This reflects the current RF science and prudent public health response that would reasonably be set for pulsed RF (ambient) exposures where people live, work and go to school. This level of RF is experienced as whole-body exposure, and can be a chronic exposure where there is wireless coverage present for voice and data transmission for cell phones, pagers and PDAs and other sources of radiofrequency radiation. Some studies and many anecdotal reports on ill health have been reported at lower levels than this; however, for the present time, it could prevent some of the most disproportionate burdens placed on the public nearest to such installations. Although this RF target level does not preclude further rollout of WI-FI technologies, we also recommend that wired alternatives to WI-FI be implemented, particularly in schools and libraries so that children are not subjected to elevated RF levels until more is understood about possible health impacts. This recommendation should be seen as an interim precautionary limit that is intended to guide preventative actions; and more conservative limits may be needed in the future.

Table 1-1   BioInitiative Report Overall Conclusions

Section 17:   Key Scientific Evidence and Public Health Policy Recommendations (continued)

- New public safety limits should be developed and implemented for ELF (50 Hz and 60 Hz electrical power frequencies). ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor.

- Guidance should be provided to electric utilities on the need to reduce ELF exposures in siting and construction of new power lines and substations. Mitigation of existing sources of ELF over 1 mG (0.1 μT) should be encouraged, particularly where children and women who are pregnant, or who may be come pregnant spend significant portions of their time.

- Requests for measurement and monitoring of ELF and RF should be provided by utilities (for power line and household ELF) and by employers (for workplace ELF and RF) ,and those who request information should receive full results of such surveys on request.

- International health organizations and agencies should issue public health advisories for those exposed to levels of ELF and RF implicated with increased risks from cancer/neurodegenerative diseases and memory/learning/immune/stress responses. These advisories should address both residential and occupational exposures.

- Reliable, unbiased information should be developed and distributed through a clearinghouse that is available to the public. Scientific, public health and policy option information should be provided for independent review at an affordable cost to the public. Research articles and prudent avoidance strategies should be made available in many languages.

- Cell phones and other wireless devices should be redesigned to operate only on speaker-phone mode or text message mode.

- Restrictions should be placed on the sale and advertising of cell phones and other wireless devices to children age 0 to 18 years.

- All countries should continue to provide wired phone service; and should be strongly discouraged from phasing it out; including pay telephones in public places.

- Manufacturers of devices that operate with wireless features should be required to carry SAR level information and warning labels on the outside packaging (not hidden inside). Wireless devices that create elevated RF levels for the user should be required to warn the user of possible adverse effects on memory and learning, cognitive function, sleep disruption and insomnia, mood disorders, balance, headache, fatigue, ringing in the ears (tinnitus), immune function, and other adverse symptoms of use.

- Warning labels on cell phones and PDAs (personal digital assistant devices) and other wireless devices are needed to alert users to excessively high ELF emissions from the switching battery pack, and require labels to list mitigation measures to reduce exposure (do not wear on or near body in “ON-Receive” position; use only with earpiece or on speaker mode, etc).

- Disclosure should be provided to the public on the location and operating characteristics of all wireless antenna sites in a fashion easily accessible to the public so informed choices can be made about where to live, shop, work and go to school. Such information should mandatorily include cumulative RF/MW exposures based on calculations from FCC OET Bulletin 65 (or equivalent) at ground level and second story level in increments of 50 feet outward from the facility to a power density of 0.1 μW/cm2 or 0.614 V/m. Signage for the public should be a mandatory condition of approval for all sites, and should be kept current.   Public agencies that approve and monitor wireless sites should require the applicant to identify locations of wireless facilities.

Table 1-1   BioInitiative Report Overall Conclusions

Section 17:   Key Scientific Evidence and Public Health Policy Recommendations (continued)

- Mobile phone - free and WI-FI-free public areas should be established in areas where the public congregates and can have a reasonable expectation of safety; including airports, public shopping, hospitals, libraries, medical clinics, convalescent homes and assisted living facilities, theatres, restaurants, parks, etc.

- Health agencies and school districts should strongly discourage or prohibit cell towers on or near (within 1000’ of) school properties, should delay any new WLAN installations in school classrooms, pre-schools and day-care facilities; and should either remove or disable existing wireless facilities, or be required to offer classrooms with no RF exposure to those families who choose not to have their children involuntarily exposed.

SECTION 1

Summary for the Public
(2014 Supplement)

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Prepared for the BioInitiative Working Group
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I. SUMMARY FOR THE PUBLIC

A. Introduction

The BioInitiative Working Group concluded in 2007 that existing public safety limits were inadequate to protect public health, and agreed that new, biologically-based public safety limits were needed five years ago. The BioInitiative Report was prepared by more than a dozen world-recognized experts in science and public health policy; and outside reviewers also contributed valuable content and perspective.

From a public health standpoint, experts reasoned that it was not in the public interest to wait. In 2007, the evidence at hand coupled with the enormous populations placed at possible risk was argued as sufficient to warrant strong precautionary measures for RFR, and lowered safety limits for ELF-EMF. The ELF recommendations were biologically-based and reflected the ELF levels consistently associated with increased risk of childhood cancer, and further incorporated a safety factor that is proportionate to others used in similar circumstances. The public health cost of doing nothing was judged to be unacceptable in 2007.

What has changed in 2012? In twenty-four technical chapters, the contributing authors discuss the content and implications of about 1800 new studies. Overall, these new studies report abnormal gene transcription (Section 5); genotoxicity and single- and double-strand DNA damage (Section 6); stress proteins because of the fractal RF-antenna like nature of DNA (Section 7); chromatin condensation and loss of DNA repair capacity in human stem cells (Sections 6 and 15); reduction in free-radical scavengers, particularly melatonin (Sections 5, 9, 13, 14, 15, 16 and 17); neurotoxicity in humans and animals (Section 9); carcinogenicity in humans (Sections 11, 12, 13, 14, 15, 16 and 17); serious impacts on human and animal sperm morphology and function (Section 18); effects on the fetus, neonate and offspring (Section 18 and 19); effects on brain and cranial bone development in the offspring of animals that are exposed to cell phone radiation during pregnancy (Sections 5 and 18); and findings in autism spectrum disorders consistent with EMF/RFR exposure. This is only a snapshot of the evidence presented in the BioInitiative 2012 updated report.

There is reinforced scientific evidence of risk from chronic exposure to low-intensity electromagnetic fields and to wireless technologies (radiofrequency radiation including microwave radiation). The levels at which effects are reported to occur is lower by hundreds of times in comparison to 2007. The range of possible health effects that are adverse with chronic exposures has broadened. There has been a big increase in the number of studies looking at the effects of cell phones (on the belt, or in the pocket of men radiating only on standby mode) and from wireless laptops on impacts to sperm quality and motility; and sperm death (fertility and reproduction). In other new studies of the fetus, infant and young child, and child-in-school – there are a dozen or more new studies of importance. There is more evidence that such exposures damage DNA, interfere with DNA repair, evidence of toxicity to the human genome (genes), more worrisome effects on the nervous system (neurology) and more and better studies on the effects of mobile phone base stations (wireless antenna facilities or cell towers) that report lower RFR levels over time can result in adverse health impacts.

Importantly, some very large studies were completed on brain tumor risk from cell phone use. The 13-country World Health Organization Interphone Final study (2010) produced evidence (although highly debated among fractious members of the research committee) that cell phone use at 10 years or longer, with approximately 1,640 hours of cumulative use of a cell and/or cordless phone approximately doubles glioma risk in adults. Gliomas are aggressive, malignant tumors where the average life-span following diagnosis is about 400 days. That brain tumors should be revealed in epidemiological studies at ONLY 10 or more years is significant; x-ray and other ionizing radiation exposures that can also cause brain tumors take nearly 15-20 years to appear making radiofrequency/microwave radiation from cell phones a very effective cancer-causing agent. Studies by Lennart Hardell and his research team at Orebro University in Sweden later showed that children who start using a mobile phone in early years have more than a 5-fold (more than a 500%) risk for developing a glioma by the time they are in the 20-29 year age group. This has significant ramifications for public health intervention.

In short order, in 2011 the World Health Organization International Agency on Cancer Research (IARC) classified radiofrequency radiation as a Group 2B Possible Human Carcinogen, joining the IARC classification of ELF-EMF that occurred in 2001. The evidence for carcinogenicity for RFR was primarily from cell phone/brain tumor studies but by IARC rules, applies to all RFR exposures (it applies to the exposure, not just to devices like cell phones or cordless phones that emit RFR).

B. Why We Care?

The stakes are very high. Exposure to electromagnetic fields (both extremely low-frequency ELF-EMF from power frequency sources like power lines and appliances; and radiofrequency radiation or RFR) has been linked to a variety of adverse health outcomes that may have significant public health consequences. The most serious health endpoints that have been reported to be associated with extremely low frequency (ELF) and/or radiofrequency radiation (RFR) include childhood and adult leukemia, childhood and adult brain tumors, and increased risk of the neurodegenerative diseases, Alzheimer’s and amyotrophic lateral sclerosis (ALS). In addition, there are reports of increased risk of breast cancer in both men and women, genotoxic effects (DNA damage, chromatin condensation, micronucleation, impaired repair of DNA damage in human stem cells), pathological leakage of the blood–brain barrier, altered immune function including increased allergic and inflammatory responses, miscarriage and some cardiovascular effects. Insomnia (sleep disruption) is reported in studies of people living in very low-intensity RF environments with WI-FI and cell tower-level exposures. Short-term effects on cognition, memory and learning, behavior, reaction time, attention and concentration, and altered brainwave activity (altered EEG) are also reported in the scientific literature. Biophysical mechanisms that may account for such effects can be found in various articles and reviews (Sage, 2012).

Traditional scientific consensus and scientific method is but one contributor to deciding when to take public health action; rather, it is one of several voices that are important in determining when new actions are warranted to protect public health. Certainly it is important, but not the exclusive purview of scientists alone to determine for all of society when changes are in the public health interest and welfare of children.

C. Do We Know Enough to Take Action

Human beings are bioelectrical systems. Our hearts and brains are regulated by internal bioelectrical signals. Environmental exposures to artificial EMFs can interact with fundamental biological processes in the human body. In some cases, this may cause discomfort, or sleep disruption, or loss of well-being (impaired mental functioning and impaired metabolism) or sometimes, maybe it is a dread disease like cancer or Alzheimer's disease. It may be interfering with one's ability to become pregnant, or to carry a child to full term, or result in brain development changes that are bad for the child. It may be these exposures play a role in causing long-term impairments to normal growth and development of children, tipping the scales away from becoming productive adults. The use of common wireless devices like wireless laptops and mobile phones requires urgent action simply because the exposures are everywhere in daily life; we need to define whether and when these exposures can damage health, or the children of the future who will be born to parents now immersed in wireless exposures.

Since World War II, the background level of EMF from electrical sources has risen exponentially, most recently by the soaring popularity of wireless technologies such as cell phones (six billion in 2011-12, up from two billion in 2006), cordless phones, WI-FI ,WiMAX and LTE networks. Some countries are moving from telephone landlines (wired) to wireless phones exclusively, forcing wireless exposures on uninformed populations around the world. These wireless exposures at the same time are now classified by the world's highest authority on cancer assessment, the World Health Organization International Agency for Research on Cancer to be a possible risk to health. Several decades of international scientific research confirm that EMFs are biologically active in animals and in humans. Now, the balance has clearly shifted to one of 'presumption of possible adverse effects' from chronic exposure. It is difficult to conclude otherwise, when the bioeffects that are clearly now occurring lead to such conditions as pathological leakage of the blood-brain barrier (allowing toxins into the brain tissues); oxidative damage to DNA and the human genome, preventing normal DNA repair in human stem cells; interfering with healthy sperm production; producing poor quality sperm or low numbers of healthy sperm, altering fetal brain development that may be fundamentally tied to epidemic rates of autism and problems in school children with memory, attention, concentration, and behavior; and leading to sleep disruptions that undercut health and healing in numerous ways.

In today's world, everyone is exposed to two types of EMFs: (1) extremely low frequency electromagnetic fields (ELF) from electrical and electronic appliances and power lines and (2) radiofrequency radiation (RFR) from wireless devices such as cell phones and cordless phones, cellular antennas and towers, and broadcast transmission towers. In this report we will use the term EMFs when referring to all electromagnetic fields in general; and the terms ELF or RFR when referring to the specific type of exposure. They are both types of non-ionizing radiation, which means that they do not have sufficient energy to break off electrons from their orbits around atoms and ionize (charge) the atoms, as do x-rays, CT scans, and other forms of ionizing radiation. A glossary and definitions are provided in this report to assist you. Some handy definitions you will probably need when reading about ELF and RF in this summary section (the language for measuring it) are shown in Section 26 – Glossary.

II. SUMMARY OF THE SCIENCE

A. Evidence for Damage to Sperm and Reproduction

Several international laboratories have replicated studies showing adverse effects on sperm quality, motility and pathology in men who use and particularly those who wear a cell phone, PDA or pager on their belt or in a pocket (See Section 18 for references including Agarwal et al, 2008; Agarwal et al, 2009; Wdowiak et al, 2007; De Iuliis et al, 2009; Fejes et al, 2005; Aitken et al, 2005; Kumar, 2012). Other studies conclude that usage of cell phones, exposure to cell phone radiation, or storage of a mobile phone close to the testes of human males affect sperm counts, motility, viability and structure (Aitken et al, 2004; Agarwal et al, 2007; Erogul et al, 2006). Animal studies have demonstrated oxidative and DNA damage, pathological changes in the testes of animals, decreased sperm mobility and viability, and other measures of deleterious damage to the male germ line (Dasdag et al, 1999; Yan et al, 2007; Otitoloju et al, 2010; Salama et al, 2008; Behari et al, 2006; Kumar et al, 2012). There are fewer animal studies that have studied effects of cell phone radiation on female fertility parameters. Panagopoulous et al (2012) report decreased ovarian development and size of ovaries, and premature cell death of ovarian follicles and nurse cells in *Drosophila melanogaster*. Gul et al (2009) reported rats exposed to stand-by level RFR (phones on but not transmitting calls) had a decrease in the number of ovarian follicles in pups born to these exposed dams. Magras and Xenos (1997) reported irreversible infertility in mice after five (5) generations of exposure to RFR at cell phone tower exposure levels of less than one microwatt per centimeter squared (µW/cm2). See Section 18 for references.

HUMAN SPERM AND THEIR DNA ARE DAMAGED
Human sperm are damaged by cell phone radiation at very low intensities (0.00034 – 0.07 µW/cm2). There is a veritable flood of new studies reporting sperm damage in humans and animals, leading to substantial concerns for fertility, reproduction and health of the offspring (unrepaired de novo mutations in sperm). Exposure levels are similar to those resulting from wearing a cell phone on the belt, or in the pants pocket, or using a wireless laptop computer on the lap. Sperm lack the ability to repair DNA damage. (Behari and Rajamani, Section 18) young child are more vulnerable than older persons are to chemicals and ionizing radiation. The US Environmental Protection Agency (EPA) proposes a 10-fold risk adjustment for the first 2 years of life exposure to carcinogens, and a 3-fold adjustment for years 3 to 5. These adjustments do not deal with fetal risk, and the possibility of extending this protection to the fetus should be examined, because of fetus' rapid organ development.

The Presidential Cancer Panel (2010) found that children "are at special risk due to their smaller body mass and rapid physical development, both of which magnify their vulnerability to known carcinogens, including radiation." The American Academy of Pediatrics, in a letter to Congressman Dennis Kucinich dated 12 December 2012 states: "Children are disproportionately affected by environmental exposures, including cell phone radiation. The differences in bone density and the amount of fluid in a child's brain compared to an adult's brain could allow children to absorb greater quantities of RF energy deeper into their brains than adults. It is essential that any new standards for cell phones or other wireless devices be based on protecting the youngest and most vulnerable populations to ensure they are safeguarded through their lifetimes."

The issue around exposure of children to RFR is of critical importance. There is overwhelming evidence that children are more vulnerable than adults to many different exposures (Sly and Carpenter, 2012), including RFR, and that the diseases of greatest concern are cancer and effects on neurodevelopment. Yet parents place RFR-emitting baby monitors in cribs, provide very young children with wireless toys, and give cell phones to young children, usually without any knowledge of the potential dangers. A growing concern is the movement to make all student computer laboratories in schools wireless. A wired computer laboratory will not increase RFR exposure, and will provide safe access to the Internet (Section, Sage and Carpenter, BioInitiative 2012 Report).

C. Evidence for Fetal and Neonatal Effects

Effects on the developing fetus from in-utero exposure to cell phone radiation have been observed in both human and animal studies since 2006. Sources of fetal and neonatal exposures of concern include cell phone radiation (both paternal use of wireless devices worn on the body and maternal use of wireless phones during pregnancy). Sources include exposure to whole-body RFR from base stations and Wi-Fi, use of wireless laptops, use of incubators for newborns with excessively high ELF-EMF levels resulting in altered heart rate variability and reduced melatonin levels in newborns, fetal exposures to MRI of the pregnant mother, and greater susceptibility to leukemia and asthma in the child where there have been maternal exposures to ELF-EMF. Divan et al (2008) found that children born to mothers who used cell phones during pregnancy develop more behavioral problems by the time they have reached school age than children whose mothers did not use cell phones during pregnancy. Children whose mothers used cell phones during pregnancy had 25% more emotional problems, 35% more hyperactivity, 49% more conduct problems and 34% more peer problems (Divan et al, 2008). Aldad et al (2012) showed that cell phone radiation significantly altered fetal brain development and produced ADHD-like behavior in the offspring of pregnant mice. Exposed mice had a dose-dependent impaired glutamatergic synaptic transmission onto Layer V pyramidal neurons of the prefrontal cortex. The authors conclude the behavioral changes were the result of altered neuronal developmental programming in utero. Offspring mice were hyperactive and had impaired memory function and behavior problems, much like the human children in Divan et al (2008). See Sections 19 and 20 for references. Fragopoulou et al (2012) reports that brain astrocyte development followed by proteomic studies is adversely affected by DECT (cordless phone radiation) and mobile phone radiation.

Fetal (in-utero) and early childhood exposures to cell phone radiation and wireless technologies in general may be a risk factor for hyperactivity, learning disorders and behavioral problems in school. Common sense measures to limit both ELF-EMF and RF EMF in these populations is needed, especially with respect to avoidable exposures like incubators that can be modified; and where education of the pregnant mother with respect to laptop computers, mobile phones and other sources of ELF-EMF and RF EMF are easily instituted.

A precautionary approach may provide the frame for decision-making where remediation actions have to be realized to prevent high exposures of children and pregnant woman.

(Bellieni and Pinto, 2012 – Section 19)

D. Evidence for Effects on Autism (Autism Spectrum Conditions)

Physicians and health care practitioners should raise the visibility of EMF/RFR as a plausible environmental factor in ASC clinical evaluations and treatment protocols. Reducing or removing EMF and wireless RFR stressors from the environment is a reasonable precautionary action given the overall weight of evidence for a link to ASCs.

Several thousand scientific studies over four decades point to serious biological effects and health harm from EMF and RFR. These studies report genotoxicity, single-and double-strand DNA damage, chromatin condensation, loss of DNA repair capacity in human stem cells, reduction in free-radical scavengers (particularly melatonin), abnormal gene transcription, neurotoxicity, carcinogenicity, damage to sperm morphology and function, effects on behavior, and effects on brain development in the fetus of human mothers that use cell phones during pregnancy. Cell phone exposure has been linked to altered fetal brain development and ADHD-like behavior in the offspring of pregnant mice.

Many disrupted physiological processes and impaired behaviors in people with ASCs closely resemble those related to biological and health effects of EMF/RFR exposure. Biomarkers and indicators of disease and their clinical symptoms have striking similarities. At the cellular and molecular level many studies of people with ASCs have identified oxidative stress and evidence of free-radical damage, as well as deficiencies of antioxidants such as glutathione. Elevated intracellular calcium in ASCs can be associated with genetic mutations but more often may be downstream of inflammation or chemical exposures. Lipid peroxidation of cell membranes, disruption of calcium metabolism, altered brain wave activity and consequent sleep, behavior and immune dysfunction, pathological leakage of critical barriers between gut and blood or blood and brain may also occur. Mitochondria may function poorly, and immune system disturbances of various kinds are common. Changes in brain and autonomic nervous system electrophysiology can be measured and seizures are far more common than in the population at large. Sleep disruption and high levels of stress are close to universal. All of these phenomena have also been documented to result from or be modulated by EMF/RFR exposure.

- • Children with existing neurological problems that include cognitive, learning, attention, memory, or behavioral problems should as much as possible be provided with wired (not wireless) learning, living and sleeping environments.
- • Special education classrooms should observe 'no wireless' conditions to reduce avoidable stressors that may impede social, academic and behavioral progress.
- • All children should reasonably be protected from the physiological stressor of significantly elevated EMF/RFR (wireless in classrooms, or home environments).
- • School districts that are now considering all-wireless learning environments should be strongly cautioned that wired environments are likely to provide better learning and teaching environments, and prevent possible adverse health consequences for both students and faculty in the long-term.
- • Monitoring of the impacts of wireless technology in learning and care environments should be performed with sophisticated measurement and data analysis techniques that are cognizant of the non-linear impacts of EMF/RFR and of data techniques most appropriate for discerning these impacts.
- • There is sufficient scientific evidence to warrant the selection of wired Internet, wired classrooms and wired learning devices, rather than making an expensive and potentially health-harming commitment to wireless devices that may have to be substituted out later.
- • Wired classrooms should reasonably be provided to all students who opt-out of wireless environments.

(Herbert and Sage, 2012 – Section 20)

The public needs to know that these risks exist, that transition to wireless should not be presumed safe, and that it is very much worth the effort to minimize exposures that still provide the benefits of technology in learning, but without the threat of health risk and development impairments to learning and behavior in the classroom.

Broader recommendations also apply, related to reducing the physiological vulnerability to exposures, reduce allostatic load and build physiological resiliency through high quality nutrition, reducing exposure to toxicants and infectious agents, and reducing stress, all of which can be implemented safely based upon presently available knowledge.

E. Evidence for Electrohypersensitivity

The contentious question of whether electrohypersensitivity exists as a medical condition and what kinds of testing might reveal biomarkers for diagnosis and treatment has been furthered by several new studies presented in Section 24 – Key Scientific Evidence and Public Health Policy Recommendations. What is evident is that a growing number of people world-wide have serious and debilitating symptoms that key to various types of EMF and RFR exposure. Of this there is little doubt. The continued massive rollout of wireless technologies, in particular the wireless 'smart' utility meter, has triggered thousands of complaints of ill-health and disabling symptoms when the installation of these meters is in close proximity to family home living spaces.

McCarty et al (2011) studied electrohypersensitivity in a patient (a female physician). The patient was unable to detect the presence or absence of EMF exposure, largely ruling out the possibility of bias. In multiple trials with the fields either on or not on, the subject experienced and reported temporal pain, feeling of unease, skipped heartbeats, muscle twitches and/or strong headache when the pulsed field (100 ms, duration at 10 Hz) was on, but no or mild symptoms when it was off. Symptoms from continuous fields were less severe than with pulsed fields. The differences between field on and sham exposure were significant at the $p < 0.05$ level. The authors conclude that electromagnetic hypersensitivity is a neurological syndrome, and statistically reliable somatic reactions can be provoked in this patient by exposure to 60-Hz electric fields at 300 volts per meter (V/m). Marino et al (2012) responded to comments on his study with McCarty saying:

> *"EMF hypersensitivity can occur as a bona fide environmentally inducible neurological syndrome. We followed an empirical approach and demonstrated a cause-and-effect relationship ($p < 0.05$) under conditions that permitted us to infer the existence of electromagnetic hypersensitivity (EHS), a novel neurological syndrome."*

The team of Sandstrom, Hansson Mild and Lyskov produced numerous papers between 1994 and 2003 involving people who are electrosensitive (See Section 24 - Lyskov et al, 1995; Lyskov et al, 1998; Sandstrom et al, 1994; Sandstrom et al, 1995;

Sandstrom et al, 1997; Sandstrom et al, 2003). Sandstrom et al (2003) presented evidence that heart rate variability is impaired in people with electrical hypersensitivity and showed disruption of the autonomic nervous system.

"EHS patients had a disturbed pattern of circadian rhythms of HRF and showed a relatively 'flat' representation of hourly-recorded spectral power of the HF component of HRV". This research team also found that "EHS patients have a dysbalance of the autonomic nervous system (ANS) regulation with a trend to hyper-sympathotonia, as measured by heart rate (HR) and electrodermal activity, and a hyperreactivity to different external physical factors, as measured by brain evoked potentials and sympathetic skin responses to visual and audio stimulation." (Lyskov et al, 2001 a,b; Sandstrom et al, 1997).

The reports referenced above provide evidence that persons who report being electrosensitive differ from others in having some abnormalities in the autonomic nervous system, reflected in measures such as heart rate variability.

F. Evidence for Effects from Cell Tower-Level RFR Exposures

Very low exposure RFR levels are associated with bioeffects and adverse health effects. At least five new cell tower studies are reporting bioeffects in the range of 0.001 to 0.05 μW/cm2 at lower levels than reported in 2007 (0.05 to 0.1 uW/cm2 was the range below which, in 2007, effects were not observed). Researchers report headaches, concentration difficulties and behavioral problems in children and adolescents; and sleep disturbances, headaches and concentration problems in adults. Public safety standards are 1,000 – 10,000 or more times higher than levels now commonly reported in mobile phone base station studies to cause bioeffects.

Since 2007, five new studies of base station level RFR at intensitites ranging from lessthan 0.001 uW/cm2 to 0.05 uW/cm2 report headaches, concentrationdifficulties and behavioral problems in children and adolescents; and sleep disturbances, headaches and concentration problems in adults.

G. Evidence for Effects on the Blood-brain Barrier (BBB)

The Lund University (Sweden) team of Leif Salford, Bertil Persson and Henrietta Nittby has done pioneering work on effects of very low level RFR on the human brain's protective lining – the barrier that protects the brain from large molecules and toxins that are in the blood.

THE BLOOD-BRAIN BARRIER IS AT RISK
The BBB is a protective barrier that prevents the flow of toxins into sensitive brain tissue. Increased permeability of the BBB caused by cell phone RFR may result in neuronal damage. Many research studies show that very low intensity exposures to RFR can affect the blood-brain barrier (BBB) (mostly animal studies). Summing up the research, it is more probable than unlikely that non-thermal EMF from cell phones and base stations do have effects upon biology. A single 2-hr exposure to cell phone radiation can result in increased leakage of the BBB, and 50 days after exposure, neuronal damage can be seen, and at the later time point also albumin leakage is demonstrated. The levels of RFR needed to affect the BBB have been shown to be as low as 0.001 W/kg, or less than holding a mobile phone at arm's length. The US FCC standard is 1.6 W/kg; the ICNIRP standard is 2 W/kg of energy (SAR) into brain tissue from cell/cordless phone use. Thus, BBB effects occur at about 1000 times lower RFR exposure levels than the US and ICNIRP limits allow.

(Salford et al, 2012 - Section 10)

H. Evidence for Effects on Brain Tumors

The Orebro University (Sweden) team led by Lennart Hardell, MD, an oncologist and medical researcher, has produced an extraordinary body of work on environmental toxins of several kinds, including the effects of radiofrequency/microwave radiation and cancer. Their 2012 work concludes:

> “Based on epidemiological studies there is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of mobile phones and cordless phones. The evidence comes mainly from two study centres, the Hardell group in Sweden and the Interphone Study Group. No consistent pattern of an increased risk is seen for meningioma. A systematic bias in the studies that explains the results would also have been the case for meningioma. The different risk pattern for tumor type strengthens the findings regarding glioma and acoustic neuroma. Meta-analyses of the Hardell group and Interphone studies show an increased risk for glioma and acoustic neuroma. Supportive evidence comes also from anatomical localisation of the tumor to the most exposed area of the brain, cumulative exposure in hours and latency time that all add to the biological relevance of an increased risk. In addition risk calculations based on estimated absorbed dose give strength to the findings. (Hardell et al, 2012 – Section 11)

> “There is reasonable basis to conclude that RF-EMFs are bioactive and have a potential to cause health impacts. There is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of wireless phones (mobile phones and cordless phones) mainly based on results from case-control studies from the Hardell group and Interphone Final Study results. Epidemiological evidence gives that RF-EMF should be classified as a human carcinogen. Based on our own research and review of other evidence the existing FCC/IEE and ICNIRP public safety limits and reference levels are not adequate to protect public health. New public health standards and limits are needed. (Hardell et al, 2012 – Section 11)

I. Evidence for Genotoxic Effects (Genotoxicity)

Genetic Damage (Genotoxicity Studies): There are at least several hundred published papers that report EMF (ELF/RFR) can affect cellular oxidative processes (oxidative damage). Increased free radical activity and changes in enzymes involved in cellular oxidative processes are the most consistent effects observed in cells and animals after EMF exposure. Aging may make an individual more susceptible to the detrimental effects of ELF EMF from oxidative damage, since anti-oxidants may decline with age. Clearly, the preponderance of genetic studies report DNA damage and failure to repair DNA damage.

> One hundred fourteen (114) new papers on genotoxic effects of RFR published between 2007 and early 2014 are profiled. Of these, 74 (65%) showed effects and 40 (35%) showed no effects. (Lai, 2014 – Section 6)
>
> Fifty nine (59) new ELF-EMF papers and two static magnetic field papers that report on genotoxic effects of ELF-EMF published between 2007 and early 2014 are profiled. Of these, 49 (83%) show effects and 10 (17%) show no effect. (Lai, 2014 – Section 6)

Factors that act directly or indirectly on the nervous system can cause morphological, chemical, or electrical changes in the nervous system that can lead to neurological effects. Both RF and ELF EMF affect neurological functions and behavior in animals and humans.

Two hundred eleven (211) new papers that report on neurological effects of RFR published between 2007 and early 2014 are profiled. Of these, 144 (68%) showed effects and 67 (32%) showed no effects.

One hundred five (105) new ELF-EMF papers (including two static field papers) that report on neurological effects of ELF-EMF published between 2007 and early 2014 are profiled. Of these, 95 (90%) show effects and 10 (10%) show no effect. (Lai, 2014 – Section 9)

K. Evidence for Cancer (Childhood Leukemia)

With overall 42 epidemiological studies published to datel power frequency ELF-EMF is among the most comprehensively studied environmental factors. Except ionizing radiation no other environmental factor has been as firmly established to increase the risk of childhood leukemia.

Sufficient evidence exists from epidemiological studies of an increased risk from exposure to EMF (power frequency ELF-EMF magnetic fields) and cannot be attributed to chance, bias or confounding. Therefore, according to the rules of IARC such exposures can be classified as a **Group 1 carcinogen (Known Carcinogen).**

There is no other risk factor identified so far for which such unlikely conditions have been put forward to postpone or deny the necessity to take steps towards exposure reduction. As one step in the direction of precaution, measures should be implemented to guarantee that exposure due to transmission and distribution lines is below an average of about 1 mG. This value is arbitrary at present and only supported by the fact that in many studies this level has been chosen as a reference. (Kundi, 2012 – Section 12)

L. Melatonin, Breast Cancer and Alzheimer's Disease

MELATONIN AND BREAST CANCER: Eleven (11) of the 13 published epidemiologic residential and occupational studies are considered to provide (positive) evidence that high ELF magnetic fields (MF) exposure can result in decreased melatonin production. The two negative studies had important deficiencies that may certainly have biased the results. There is sufficient evidence to conclude that long-term relatively high ELF MF exposure can result in a decrease in melatonin production. It has not been determined to what extent personal characteristics, e.g., medications, interact with ELF MF exposure in decreasing melatonin production.

There is sufficient evidence to conclude that long-term relatively high ELF MF exposure can result in a decrease in melatonin production, which may increase risk for breast cancer. It has not been determined to what extent personal characteristics, e.g., medications, interact with ELF MF exposure in decreasing melatonin production. New research indicates that ELF MF exposure, in vitro, can significantly decrease melatonin activity through effects on MT1, an important melatonin receptor. Five longitudinal studies have now been conducted of low melatonin production as a risk factor for breast cancer. There is increasingly strong longitudinal evidence that low melatonin production is a risk factor for at least post-menopausal breast cancer.

(Davanipour and Sobel, 2012 – Section 13)

ALZHEIMER'S DISEASE: There is now evidence that a) high levels of peripheral amyloid beta are a risk factor for AD, and b) medium to high ELF MF exposure can increase peripheral amyloid beta. High brain levels of amyloid beta are also a risk factor for AD and medium to high ELF MF exposure to brain cells likely also increases these cells' production of amyloid beta. There is considerable in vitro and animal evidence that melatonin protects against AD. Therefore it is certainly possible that low levels of melatonin production are associated with an increase in the risk of AD.

There is strong epidemiologic evidence that exposure to ELF MF is a risk factor for AD. There are now twelve (12) studies of ELF MF exposure and AD or dementia. Nine (9) of these studies are considered positive and three (3) are considered negative. The three negative studies have serious deficiencies in ELF MF exposure classification that results in subjects with rather low exposure being considered as having significant exposure. There are insufficient studies to formulate an opinion as to whether radiofrequency MF exposure is a risk or protective factor for AD.

There is now evidence that (i) high levels of peripheral amyloid beta are a risk factor for AD and (ii) medium to high ELF MF exposure can increase peripheral amyloid beta. High brain levels of amyloid beta are also a risk factor for AD and medium to high ELF MF exposure to brain cells likely also increases these cells' production of amyloid beta.

There is considerable in vitro and animal evidence that melatonin protects against AD. Therefore it is certainly possible that low levels of melatonin production are associated with an increase in the risk of AD.

(Davanipour and Sobel, 2012 – Section 13)

M. Stress, Stress Proteins and DNA as a Fractal Antenna

Any agent (EMF, ionizing radiation, chemicals, heavy metals, heat and other factors) that continuously generates stress proteins is not adaptive, and is harmful, if it is a constant provocation. The work of Martin Blank and Reba Goodman of Columbia University has established that stress proteins are produced by ELF-EMF and RFR at levels far below what current safety standards allow. Further, they think DNA is actually a very good fractal RF-antenna which is very sensitive to low doses of EMF, and may induce the cellular processes that result in chronic 'unrelenting' stress. That daily environmental levels of ELF-EMF and RFR can and do throw the human body into stress protein response mode (out of homeostasis) is a fundamental and continuous insult. Chronic exposures can then result in chronic ill-health**.**

> *"It appears that the DNA molecule is particularly vulnerable to damage by EMF because of the coiled-coil configuration of the compacted molecule in the nucleus. The unusual structure endows it with the self similarity of a fractal antenna and the resulting sensitivity to a wide range of frequencies. The greater reactivity of DNA with EMF, along with a vulnerability to damage,*

> *underscores the urgent need to revise EMF exposure standards in order to protect the public. Recent studies have also exploited the properties of stress proteins to devise therapies for limiting oxidative damage and reducing loss of muscle strength associated with aging." (Blank, 2012- Section 7)*

- DNA acts as a 'fractal antenna' for EMF and RFR. The coiled-coil structure of DNA in the nucleus makes the molecule react like a fractal antenna to a wide range of frequencies.
- The structure makes DNA particularly vulnerable to EMF damage.
- The mechanism involves direct interaction of EMF with the DNA molecule (claims that there are no known mechanisms of interaction are patently false).
- Many EMF frequencies in the environment can and do cause DNA changes.
- The EMF-activated cellular stress response is an effective protective mechanism for cells exposed to a wide range of EMF frequencies.
- EMF stimulates stress proteins (indicating an assault on the cell).
- EMF efficiently harms cells at billions of times lower levels than conventional heating.
- Safety standards based on heating are irrelevant to protect against EMF-levels of exposure. There is an urgent need to revise EMF exposure standards. Research has shown thresholds are very low (safety standards must be reduced to limit biological responses). Biologically-based safety standards could be developed from the research on the stress response. (Blank, 2012 – Section 7).

N. Effects of Weak-Field Interactions on Non-Linear Biological Oscillators and Synchronized Neural Activity:

A unifying hypothesis for a plausible biological mechanism to account for very weak field EMF bioeffects other than cancer may lie with weak field interactions of pulsed RFR and ELF-modulated RFR as disrupters of synchronized neural activity. Electrical rhythms in our brains can be influenced by external signals. This is consistent with established weak field effects on coupled biological oscillators in living tissues. Biological systems of the heart, brain and gut are dependent on the cooperative actions of cells that function according to principles of non-linear, coupled biological oscillations for their synchrony, and are dependent on exquisitely timed cues from the environment at vanishingly small levels (Buzsaki, 2006; Strogatz, 2003). The key to synchronization is the joint actions of cells that co-operate electrically and link populations of biological oscillators that couple together in large arrays and synchronize spontaneously. Synchronous biological oscillations in cells (pacemaker cells) can be disrupted by artificial, exogenous environmental signals, resulting in desynchronization of neural activity that regulates critical functions (including metabolism) in the brain, gut and heart and circadian rhythms governing sleep and hormone cycles (Strogatz, 1987). The brain contains a population of oscillators with distributed natural frequencies, which pull one another into synchrony (the circadian pacemaker cells). Strogatz has addressed the unifying mathematics of biological cycles and external factors disrupt these cycles (Strogatz, 2001, 2003)

> *"Rhythms can be altered by a wide variety of agents and that these perturbations must seriously alter brain performance." (Busaki, 2006)*

III. EMF EXPOSURE AND PRUDENT PUBLIC HEALTH PLANNING

Chronic exposure to low-intensity RFR and to ELF-modulated RFR at today's environmental levels in many cities will exceed thresholds for increased risk of many diseases and causes of death (Sage and Huttunen, 2012). RFR exposures in daily life alter homeostasis in human beings. These exposures can alter and damage genes, trigger epigenetic changes to gene expression and cause de novo mutations that prevent genetic recovery and healing mechanisms. These exposures may interfere with normal cardiac and brain function; alter circadian rhythms that regulate sleep, healing, and hormone balance; impair short-term memory, concentration, learning and behavior; provoke aberrant immune, allergic and inflammatory responses in tissues; alter brain metabolism; increase risks for reproductive failure (damage sperm and increase miscarriage risk); and cause cells to produce stress proteins. Exposures now common in home and school environments are likely to be physiologically addictive and the effects are particularly serious in the young (Sage and Huttunen, 2012).

RECOMMENDED ACTIONS

A. Defining Preventative Actions for Reduction in RFR Exposures

ELF-EMF and RFR are Classified as Possible Cancer-causing Agents – Why Are Governments Not Acting?

The World Health Organization International Agency for Research on Cancer has classified wireless radiofrequency as a Possible Human Carcinogen (May, 2011)*. The designation applies to low-intensity RFR in general, covering all RFR-emitting devices and exposure sources (cell and cordless phones, Wi-Fi, wireless laptops, wireless hotspots, electronic baby monitors, wireless classroom access points, wireless antenna facilities). The IARC Panel could have chosen to classify RFR as a Group 4 – Not A Carcinogen if the evidence was clear that RFR is not a cancer-causing agent. It could also have found a Group 3 designation was a good interim choice (Insufficient Evidence). IARC did neither.

New Safety Limits Must Be Established – Health Agencies Should Act Now

Existing public safety limits (FCC and ICNIRP public safety limits) do not sufficiently protect public health against chronic exposure from very low-intensity exposures. If no mid-course corrections are made to existing and outdated safety limits, such delay will magnify the public health impacts with even more applications of wireless-enabled technologies exposing even greater populations around the world in daily life.

Scientific Benchmarks for Harm Plus Safety Margins = New Safety Limits that are Valid

Health agencies and regulatory agencies that set public safety standards for ELF-EMF and RFR should act now to adopt new, biologically-relevant safety limits that key to the lowest scientific benchmarks for harm coming from the recent studies, plus a lower safety margin. Existing public safety limits are too high by several orders of magnitude, if prevention of bioeffects and resulting adverse health effects are to be minimized or eliminated. Most safety standards are a thousand times or more too high to protect healthy populations, and even less effective in protecting sensitive subpopulations.

Sensitive Populations Must Be Protected

Safety standards for sensitive populations will more likely need to be set at lower levels than for healthy adult populations. Sensitive populations include the developing fetus, the infant, children, the elderly, those with pre-existing chronic diseases, and those with developed electrical sensitivity (EHS).

Protecting New Life – Infants and Children

Strong precautionary action and clear public health warnings are warranted immediately to help prevent a global epidemic of brain tumors resulting from the use of wireless devices (mobile phones and cordless phones). Commonsense measures to limit both ELF-EMF and RFR in the fetus and newborn infant (sensitive populations) are needed, especially with respect to avoidable exposures like baby monitors in the crib and baby isolettes (incubators) in hospitals that can be modified; and where education of the pregnant mother with respect to laptop computers, mobile phones and other sources of ELF-EMF and RFR are easily instituted.

Wireless laptops and other wireless devices should be strongly discouraged in schools for children of all ages.

Standard of Evidence for Judging the Science

The standard of evidence for judging the scientific evidence should be based on good public health principles rather than demanding scientific certainty before actions are taken.

Wireless Warnings for All

The continued rollout of wireless technologies and devices puts global public health at risk from unrestricted wireless commerce unless new, and far lower exposure limits and strong precautionary warnings for their use are implemented.

EMF and RFR are Preventable Toxic Exposures

We have the knowledge and means to save global populations from multi-generational adverse health consequences by reducing both ELF and RFR exposures. Proactive and immediate measures to reduce unnecessary EMF exposures will lower disease burden and rates of premature death.

B. Defining New ‘Effect Level’ for RFR

Section 24 concludes that RFR ‘effect levels’ for bioeffects and adverse health effects justify new and lower precautionary target levels for RFR exposure. New epidemiological and laboratory studies are finding effects on humans at lower exposure levels where studies are of longer duration (chronic exposure studies). Real-world experience is revealing worrisome evidence that sperm may be damaged by cell phones even on stand-by mode; and people can be adversely affected by placing new wireless pulsed RFR transmitters (utility meters on the sides or interiors of homes), even when the time-weighted average for RFR is miniscule in both cases.

There is increasing reason to believe that the critical factor for biologic significance is the intermittent pulse of RF, not the time-averaged SAR. For example, Hansson Mild et al, (2012) concluded there could be no effect on sleep and testicular function from a GSM mobile phone because the "*exposure in stand-by mode can be considered negligible*". It may be that we, as a species, are more susceptible than we thought to intermittent, very low-intensity pulsed RFR signals that can interact with critical activities in living tissues. It is a mistake to conclude that the effect does not exist because we cannot explain HOW it is happening or it upsets our mental construct of how things should work.

This highlights the serious limitation of not taking the nature of the pulsed RFR signal (high intensity but intermittent, microsecond pulses of RFR) into account in the safety standards. This kind of signal is biologically active. Even if it is essentially mathematically invisible when the individual RFR pulses are time-averaged, it is apparently NOT invisible to the human body and its proper biological functioning.

For these reasons, and in light of parallel scientific work on non-linear biological oscillators including the accepted mathematics in this branch of science regarding coupled oscillators (Bezsaki, 2006; Strogatz, 2001, 2003), it is essential to think forward about the ramifications of shifting national energy strategies toward ubiquitous wireless systems. And, it is essential to re-think safety standards to take into account the exquisite sensitivity of biological systems and tissue interactions where the exposures are pulsed and cumulatively insignificant over time-scale averaging, but highly relevant to body processes and functioning. If it is true that weak-field effects have control elements over synchronous activity of neurons in the brain, and other pacemaker cells and tissues in the heart and gut that drive essential metabolic pathways as a result, then this will go far in explaining why living tissues are apparently so reactive to very small inputs of pulsed RFR, and lead to better understanding of what is required for new, biologically-based public exposure standards.

A reduction from the BioInitiative 2007 recommendation of 0.1 uW/cm2 (or one-tenth of a microwatt per square centimeter) for cumulative outdoor RFR down to something three orders of magnitude lower (in the low nanowatt per square centimeter range) is justified on a public health basis. We use the new scientific evidence documented in this Report to identify 'effect levels' and then apply one or more reduction factors to provide a safety margin. A cautionary target level for cumulative, outdoor pulsed RFR exposures for ambient wireless that could be applied to RFR sources from cell tower antennas, Wi-Fi, WiMAX and other similar sources is proposed. Research is needed to determine what is biologically damaging about intermittent pulses of RFR, and how to provide for protection in safety limits against it. With this knowledge it might be feasible to recommend a higher time-averaged number.

A scientific benchmark of 0.003 uW/cm2 or three nanowatts per centimeter squared for 'lowest observed effect level' for RFR is based on mobile phone base station-level studies. Applying a ten-fold reduction to compensate for the lack of long-term exposure (to provide a safety buffer for chronic exposure, if needed) or for children as a sensitive subpopulation (if studies are on adults, not children) yields a 300 to 600 picowatts per square centimeter precautionary action level. This equates to a 0.3 nanowatts to 0.6 nanowatts per square centimeter as a reasonable, precautionary action level for chronic exposure to pulsed RFR. Even so, these levels may need to change in the future, as new and better studies are completed. This is what the authors said in 2007 (Carpenter and Sage, 2007, BioInitiative Report) and it remains true today in 2012.

We leave room for future studies that may lower or raise today's observed 'effects levels' and should be prepared to accept new information as a guide for new precautionary action.

BIOINITIATIVE 2012 - CONCLUSIONS Table 1-1

(Genetics and Neurological Effects Updated March 2014)

Overall, more than 1800 or so new studies report abnormal gene transcription (Section 5); genotoxicity and single-and double-strand DNA damage (Section 6); stress proteins because of the fractal RF-antenna like nature of DNA (Section 7); chromatin condensation and loss of DNA repair capacity in human stem cells (Sections 6 and 15); reduction in free-radical scavengers - particularly melatonin (Sections 5, 9, 13, 14, 15, 16 and 17); neurotoxicity in humans and animals (Section 9), carcinogenicity in humans (Sections 11, 12, 13, 14, 15, 16 and 17); serious impacts on human and animal sperm morphology and function (Section 18); effects on offspring behavior (Section 18, 19 and 20); and effects on brain and cranial bone development in the offspring of animals that are exposed to cell phone radiation during pregnancy (Sections 5 and 18). This is only a snapshot of the evidence presented in the BioInitiative 2012 updated report.

BIOEFFECTS ARE CLEARLY ESTABLISHED

Bioeffects are clearly established and occur at very low levels of exposure to electromagnetic fields and radiofrequency radiation. Bioeffects can occur in the first few minutes at levels associated with cell and cordless phone use. Bioeffects can also occur from just minutes of exposure to mobile phone masts (cell towers), WI-FI, and wireless utility ‘smart’ meters that produce whole-body exposure. Chronic base station level exposures can result in illness.

BIOEFFECTS WITH CHRONIC EXPOSURES CAN REASONABLY BE PRESUMED TO RESULT IN ADVERSE HEALTH EFFECTS

Many of these bioeffects can reasonably be presumed to result in adverse health effects if the exposures are prolonged or chronic. This is because they interfere with normal body processes (disrupt homeostasis), prevent the body from healing damaged DNA, produce immune system imbalances, metabolic disruption and lower resilience to disease across multiple pathways. Essential body processes can eventually be disabled by incessant external stresses (from system-wide electrophysiological interference) and lead to pervasive impairment of metabolic and reproductive functions.

LOW EXPOSURE LEVELS ARE ASSOCIATED WITH BIOEFFECTS AND ADVERSE HEALTH EFFECTS AT CELL TOWER RFR EXPOSURE LEVELS

At least five new cell tower studies are reporting bioeffects in the range of 0.003 to 0.05 μW/cm2 at lower levels than reported in 2007 (0.05 to 0.1 uW/cm2 was the range below which, in 2007, effects were not observed). Researchers report headaches, concentration difficulties and behavioral problems in children and adolescents; and sleep disturbances, headaches and concentration problems in adults. Public safety standards are 1,000 – 10,000 or more times higher than levels now commonly reported in mobile phone base station studies to cause bioeffects.

EVIDENCE FOR FERTILITY AND REPRODUCTION EFFECTS: HUMAN SPERM AND THEIR DNA ARE DAMAGED

Human sperm are damaged by cell phone radiation at very low intensities in the low microwatt and nanowatt/cm2 range (0.00034 – 0.07 uW/cm2). There is a veritable flood of new studies reporting sperm damage in humans and animals, leading to substantial concerns for fertility, reproduction and health of the offspring (unrepaired de novo mutations in sperm). Exposure levels are similar to those resulting from wearing a cell phone on the belt, or in the pants pocket, or using a wireless laptop computer on the lap. Sperm lack the ability to repair DNA damage.

Studies of human sperm show genetic (DNA) damage from cell phones on standby mode and wireless laptop use. Impaired sperm quality, motility and viability occur at exposures of 0.00034 uW/cm2 to 0.07 uW/cm2 with a resultant reduction in human male fertility. Sperm cannot repair DNA damage.

Several international laboratories have replicated studies showing adverse effects on sperm quality, motility and pathology in men who use and particularly those who wear a cell phone, PDA or pager on their belt or in a pocket (Agarwal et al, 2008; Agarwal et al, 2009; Wdowiak et al, 2007; De Iuliis et al, 2009; Fejes et al, 2005; Aitken et al, 2005; Kumar, 2012). Other studies conclude that usage of cell phones, exposure to cell phone radiation, or storage of a mobile phone close to the testes of human males affect sperm counts, motility, viability and structure (Aitken et al, 2004; Agarwal et al, 2007; Erogul et al., 2006). Animal studies have demonstrated oxidative and DNA damage, pathological changes in the testes of animals, decreased sperm mobility and viability, and other measures of deleterious damage to the male germ line (Dasdag et al, 1999; Yan et al, 2007; Otitoloju et al, 2010; Salama et al, 2008; Behari et al, 2006; Kumar et al, 2012). There are fewer animal studies that have studied effects of cell phone radiation on female fertility parameters. Panagopoulous et al. 2012 report decreased ovarian development and size of ovaries, and premature cell death of ovarian follicles and nurse cells in *Drosophila melanogaster*. Gul et al (2009) report rats exposed to stand-by level RFR (phones on but not transmitting calls) caused decrease in the number of ovarian follicles in pups born to these exposed dams. Magras and Xenos (1997) reported irreversible infertility in mice after five (5) generations of exposure to RFR at cell phone tower exposure levels of less than one microwatt per centimeter squared (μW/cm2).

EVIDENCE THAT CHILDREN ARE MORE VULNERABLE

There is good evidence to suggest that many toxic exposures to the fetus and very young child have especially detrimental consequences depending on when they occur during critical phases of growth and development (time windows of critical development), where such exposures may lay the seeds of health harm that develops even decades later. Existing FCC and ICNIRP public safety limits seem to be not sufficiently protective of public health, in particular for the young (embryo, fetus, neonate, very young child).

The Presidential Cancer Panel (2010) found that children '*are at special risk due to their smaller body mass and rapid physical development, both of which magnify their vulnerability to known carcinogens, including radiation.*'

The American Academy of Pediatrics, in a letter to Congressman Dennis Kucinich dated 12 December 2012 states "*Children are disproportionately affected by environmental exposures, including cell phone radiation. The differences in bone density and the amount of fluid in a child's brain compared to an adult's brain could allow children to absorb greater quantities of RF energy deeper into their brains than adults. It is essential that any new standards for cell phones or other wireless devices be based on protecting the youngest and most vulnerable populations to ensure thay are safeguarded through their lifetimes.*"

FETAL AND NEONATAL EFFECTS OF EMF

Fetal (*in-utero*) and early childhood exposures to cell phone radiation and wireless technologies in general may be a risk factor for hyperactivity, learning disorders and behavioral problems in school.

Fetal Development Studies: Effects on the developing fetus from *in-utero* exposure to cell phone radiation have been observed in both human and animal studies since 2006. Divan et al (2008) found that children born of mothers who used cell phones during pregnancy develop more behavioral problems by the time they have reached school age than children whose mothers did not use cell phones during pregnancy. Children whose mothers used cell phones during pregnancy had 25% more emotional problems, 35% more hyperactivity, 49% more conduct problems and 34% more peer problems
(Divan et al., 2008).

Common sense measures to limit both ELF-EMF and RF EMF in these populations is needed, especially with respect to avoidable exposures like incubators that can be modified; and where education of the pregnant mother with respect to laptop computers, mobile phones and other sources of ELF-EMF and RF EMF are easily instituted.

Sources of fetal and neonatal exposures of concern include cell phone radiation (both paternal use of wireless devices worn on the body and maternal use of wireless phones during pregnancy). Exposure to whole-body RFR from base stations and WI-FI, use of wireless laptops, use of incubators for newborns with excessively high ELF-EMF levels resulting in altered heart rate variability and reduced melatonin levels in newborns, fetal exposures to MRI of the pregnant mother, and greater susceptibility to leukemia and asthma in the child where there have been maternal exposures to ELF-EMF.

A precautionary approach may provide the frame for decision-making where remediation actions have to be realized to prevent high exposures of children and pregnant woman.

(Bellieni and Pinto, 2012 – Section 19)

EMF/RFR AS A PLAUSIBLE BIOLGICAL MECHANISM FOR AUTISM (ASD)

- Children with existing neurological problems that include cognitive, learning, attention, memory, or behavioral problems should as much as possible be provided with wired (not wireless) learning, living and sleeping environments,
- Special education classrooms should observe 'no wireless' conditions to reduce avoidable stressors that may impede social, academic and behavioral progress.
- All children should reasonably be protected from the physiological stressor of significantly elevated EMF/RFR (wireless in classrooms, or home environments).
- School districts that are now considering all-wireless learning environments should be strongly cautioned that wired environments are likely to provide better learning and teaching environments, and prevent possible adverse health consequences for both students and faculty in the long-term.
- Monitoring of the impacts of wireless technology in learning and care environments should be performed with sophisticated measurement and data analysis techniques that are cognizant of the non-linear impacts of EMF/RFR and of data techniques most appropriate for discerning these impacts.
- There is sufficient scientific evidence to warrant the selection of wired internet, wired classrooms and wired learning devices, rather than making an expensive and potentially health-harming commitment to wireless devices that may have to be substituted out later, and
- Wired classrooms should reasonably be provided to all students who opt-out of wireless environments.

(Herbert and Sage, 2012 – Section 20)

Many disrupted physiological processes and impaired behaviors in people with ASDs closely resemble those related to biological and health effects of EMF/RFR exposure. Biomarkers and indicators of disease and their clinical symptoms have striking similarities. Broadly speaking, these types of phenomena can fall into one or more of several classes: a) alteration of genes or gene expression, b) induction of change in brain or organismic development, c) alteration of phenomena modulating systemic and brain function on an ongoing basis throughout the life course (which can include systemic pathophysiology as well as brain-based changes), and d) evidence of functional alteration in domains such as behavior, social interaction and attention known to be challenged in ASD.

Several thousand scientific studies over four decades point to serious biological effects and health harm from EMF and RFR. These studies report genotoxicity, single-and double-strand DNA damage, chromatin condensation, loss of DNA repair capacity in human stem cells, reduction in free-radical scavengers (particularly melatonin), abnormal gene transcription, neurotoxicity, carcinogenicity, damage to sperm morphology and function, effects on behavior, and effects on brain development in the fetus of human mothers that use cell phones during pregnancy. Cell phone exposure has been linked to altered fetal brain development and ADHD-like behavior in the offspring of pregnant mice.

Reducing life-long health risks begins in the earliest stages of embryonic and fetal development, is accelerated for the infant and very young child compared to adults, and is not complete in young people (as far as brain and nervous system maturation) until the early 20’s. Windows of critical development mean that risk factors once laid down in the cells, or in epigenetic changes in the genome may have grave and life-long consequences for health or illness for every individual.

All relevant environmental conditions, including EMF and RFR, which can degrade the human genome, and impair normal health and development of species including homo sapiens, should be given weight in defining and implementing prudent, precautionary actions to protect public health.

Allostatic load in autism and autistic decompensation - we may be at a tipping point that can be pushed back by removing unnecessary stressors like EMF/RFR and building resilience.

The consequence of ignoring clear evidence of large-scale health risks to global populations, when the risk factors are largely avoidable or preventable is too high a risk to take. With the epidemic of autism (ASD) putting the welfare of children, and their families in peril at a rate of one family in 88, the rate still increasing annually, we cannot afford to ignore this body of evidence. The public needs to know that these risks exist, that transition to wireless should not be presumed safe, and that it is very much worth the effort to minimize exposures that still provide the benefits of technology in learning, but without the threat of health risk and development impairments to learning and behavior in the classroom.

(Herbert and Sage, 2012 – Section 20)

THE BLOOD-BRAIN BARRIER IS AT RISK

The BBB is a protective barrier that prevents the flow of toxins into sensitive brain tissue. Increased permeability of the BBB caused by cell phone RFR may result in neuronal damage. Many research studies show that very low intensity exposures to RFR can affect the blood-brain barrier (BBB) (mostly animal studies). Summing up the research, it is more probable than unlikely that non-thermal EMF from cell phones and base stations do have effects upon biology. A single 2-hr exposure to cell phone radiation can result in increased leakage of the BBB, and 50 days after exposure, neuronal damage can be seen, and at the later time point also albumin leakage is demonstrated. *The levels of RFR needed to affect the BBB have been shown to be as low as 0.001 W/kg, or less than holding a mobile phone at arm's length. The US FCC standard is 1.6 W/kg; the ICNIRP standard is 2 W/kg of energy (SAR) into brain tissue from cell/cordless phone use. Thus, BBB effects occur at about 1000 times lower RFR exposure levels than the US and ICNIRP limits allow.* (Salford et al, 2012 - Section 10)

If the blood-brain barrier is vulnerable to serious and on-going damage from wireless exposures, then we should perhaps also be looking at the blood-ocular barrier (that protects the eyes), the blood-placenta barrier (that protects the developing fetus) and the blood-gut barrier (that protects proper digestion and nutrition), and the blood-testes barrier (that protects developing sperm) to see if they too can be damaged by RFR.

EPIDEMIOLOGICAL STUDIES CONSISTENTLY SHOW ELEVATIONS IN RISK OF BRAIN CANCERS

Brain Tumors: There is a consistent pattern of increased risk of glioma and acoustic neuroma associated with use of mobile phones and cordless phones.

"Based on epidemiological studies there is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of mobile phones and cordless phones. The evidence comes mainly from two study centres, the Hardell group in Sweden and the Interphone Study Group. No consistent pattern of an increased risk is seen for meningioma. A systematic bias in the studies that explains the results would also have been the case for meningioma. The different risk pattern for tumor type strengthens the findings regarding glioma and acoustic neuroma. Meta-analyses of the Hardell group and Interphone studies show an increased risk for glioma and acoustic neuroma. Supportive evidence comes also from anatomical localisation of the tumor to the most exposed area of the brain, cumulative exposure in hours and latency time that all add to the biological relevance of an increased risk. In addition risk calculations based on estimated absorbed dose give strength to the findings.

"There is reasonable basis to conclude that RF-EMFs are bioactive and have a potential to cause health impacts. There is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of wireless phones (mobile phones and cordless phones) mainly based on results from case-control studies from the Hardell group and Interphone Final Study results. Epidemiological evidence gives that RF-EMF should be classified as a human carcinogen.

Based on our own research and review of other evidence the existing FCC/IEE and ICNIRP public safety limits and reference levels are not adequate to protect public health. New public health standards and limits are needed.

(Hardell et al, 2012 –Section 11)

EVIDENCE FOR GENETIC EFFECTS (Updated March 2014)

One hundred fourteen (114) new papers on genotoxic effects of RFR published between 2007 and early 2014 are profiled. Of these, 74 (65%) showed effects and 40 (35%) showed no effects.

Fifty nine (59) new ELF-EMF papers and two static magnetic field papers that report on genotoxic effects of ELF-EMF between 2007 and early 2014 are profiled. Of these, 49 (83%) show effects and 10 (17%) show no effect.

(Lai, 2014 – Section 6)

EVIDENCE FOR NEUROLOGICAL EFFECTS (Updated March 2014)

Two hundred eleven (211) new papers that report on neurological effects of RFR published between 2007 and early 2014 are profiled. Of these, 144 (68%) showed effects and 67 (32%) showed no effects.

One hundred five (105) new ELF-EMF papers (including two static field papers) that report on neurological-effects of ELF-EMF published between 2007 and early 2014 are profiled. Of these, 95 (90%) show effects and 10 (10%) show no effect. (Lai, 2014 – Section 9)

EVIDENCE FOR CHILDHOOD CANCERS (LEUKEMIA)

With overall 42 epidemiological studies published to date power frequency EMFs are among the most comprehensively studied environmental factors. Except ionizing radiation no other environmental factor has been as firmly established to increase the risk of childhood leukemia. Sufficient evidence from epidemiological studies of an increased risk from exposure to EMF (power frequency magnetic fields) that cannot be attributed to chance, bias or confounding. Therefore, according to the rules of IARC such exposures can be classified as a **Group 1 carcinogen (Known Carcinogen).**

There is no other risk factor identified so far for which such unlikely conditions have been put forward to postpone or deny the necessity to take steps towards exposure reduction. As one step in the direction of precaution, measures should be implemented to guarantee that exposure due to transmission and distribution lines is below an average of about 1 mG. This value is arbitrary at present and only supported by the fact that in many studies this level has been chosen as a reference.

Base-station level RFR at levels ranging from less than 0.001 uW/cm2 to 0.05 uW/cm2. In 5 new studies since 2007, researchers report headaches, concentration difficulties and behavioral problems in children and adolescents; and sleep disturbances, headaches and concentration problems in adults.

MELATONIN, BREAST CANCER AND ALZHEIMER'S DISEASE

MELATONIN AND BREAST CANCER

Conclusion: Eleven (11) of the 13 published epidemiologic residential and occupational studies are considered to provide (positive) evidence that high ELF MF exposure can result in decreased melatonin production. The two negative studies had important deficiencies that may certainly have biased the results. There is sufficient evidence to conclude that long-term relatively high ELF MF exposure can result in a decrease in melatonin production. It has not been determined to what extent personal characteristics, e.g., medications, interact with ELF MF exposure in decreasing melatonin production

Conclusion: New research indicates that ELF MF exposure, in vitro, can significantly decrease melatonin activity through effects on MT1, an important melatonin receptor.

(Davanipour and Sobel, 2012 – Section 13)

ALZHEIMER'S DISEASE

There is strong epidemiologic evidence that exposure to ELF MF is a risk factor for AD. There are now twelve (12) studies of ELF MF exposure and AD or dementia which . Nine (9) of these studies are considered positive and three (3) are considered negative. The three negative studies have serious deficiencies in ELF MF exposure classification that results in subjects with rather low exposure being considered as having significant exposure. There are insufficient studies to formulate an opinion as to whether radiofrequency MF exposure is a risk or protective factor for AD.

There is now evidence that (i) high levels of peripheral amyloid beta are a risk factor for AD and (ii) medium to high ELF MF exposure can increase peripheral amyloid beta. High brain levels of amyloid beta are also a risk factor for AD and medium to high ELF MF exposure to brain cells likely also increases these cells' production of amyloid beta.

There is considerable in vitro and animal evidence that melatonin protects against AD. Therefore it is certainly possible that low levels of melatonin production are associated with an increase in the risk of AD.

(Davanipour and Sobel, 2012 – Section 13)

STRESS PROTEINS AND DNA AS A FRACTAL ANTENNA FOR RFR

DNA acts as a ‘fractal antenna’ for EMF and RFR.

The coiled-coil structure of DNA in the nucleus makes the molecule react like a fractal antenna to a wide range of frequencies.

The structure makes DNA particularly vulnerable to EMF damage.

The mechanism involves direct interaction of EMF with the DNA molecule (claims that there are no known mechanisms of interaction are patently false)

Many EMF frequencies in the environment can and do cause DNA changes.

The EMF-activated cellular stress response is an effective protective mechanism for cells exposed to a wide range of EMF frequencies.

EMF stimulates stress proteins (indicating an assault on the cell).

EMF efficiently harms cells at a billion times lower levels than conventional heating.
Blank, 2012 – Section 7)

Safety standards based on heating are irrelevant to protect against EMF-levels of exposure. There is an urgent need to revise EMF exposure standards. Research has shown thresholds are very low (safety standards must be reduced to limit biological responses). Biologically-based EMF safety standards could be developed from the research on the stress response. (Blank, 2012 – Section 7)

EVIDENCE FOR DISRUPTION OF THE MODULATING SIGNAL
HUMAN STEM CELL DNA DOES NOT ADAPT OR REPAIR

Human stem cells do not adapt to chronic exposures to non-thermal microwave (cannot repair damaged DNA), and damage to DNA in genes in other cells generally do not repair as efficiently. (Belyaev, 2012 – Section 15)

Non-thermal effects of microwaves depend on variety of biological and physical parameters that should be taken into account in setting the safety standards. Emerging evidence suggests that the SAR concept, which has been widely adopted for safety standards, is not useful alone for the evaluation of health risks from non-thermal microwave of mobile communication. Other parameters of exposure, such as frequency, modulation, duration, and dose should be taken into account.

Lower intensities are not always less harmful; they may be more harmful.

Intensity windows exist, where bioeffects are much more powerful.

A linear, dose-response relationship test is probably invalid for testing of RFR and EMF (as is done in chemicals testing for toxicity).

Resonant frequencies may result in biological effects at very low intensities comparable to base station (cell tower) and other microwave sources used in mobile communications. These exposures can cause health risk. The current safety standards are insufficient to protect from non-thermal microwave effects.

The data about the effects of microwave at super-low intensities and significant role of duration of exposure in these effects along with the data showing that adverse effects of non-thermal microwave from GSM/UMTS mobile phones depend on carrier frequency and type of the microwave signal suggest that microwave from base-stations/masts, wireless routers, WI-FI and other wireless devices and exposures in common use today can also produce adverse effects at prolonged durations of exposure.

Most of the real signals that are in use in mobile communication have not been tested so far. Very little research has been done with real signals and for durations and intermittences of exposure that are relevant to chronic exposures from mobile communication. In some studies, so-called “mobile communication-like” signals were investigated that in fact were different from the real exposures in such important aspects as intensity, carrier frequency, modulation, polarization, duration and intermittence.

New standards should be developed based on knowledge of mechanisms of non-thermal effects. Importantly, because the signals of mobile communication are completely replaced by other signals faster then once per 10 years, duration comparable with latent period, epidemiologic studies cannot provide basement for cancer risk assessment from upcoming new signals.

In many cases, because of ELF modulation and additional ELF fields created by the microwave sources, for example by mobile phones, it is difficult to distinguish the effects of exposures to ELF and microwave. Therefore, these combined exposures and their possible cancer risks should be considered in combination.

As far as different types of microwave signals (carrier frequency, modulation, polarization, far and near field, intermittence, coherence, *etc.*) may produce different effects, cancer risks should ideally be estimated for each microwave signal separately.

The Precautionary Principle should be implemented while new standards are in progress.

It should be anticipated that some part of the human population, such as children, pregnant women and groups of hypersensitive persons could be especially sensitive to the non-thermal microwave exposures.
(Belyaev, 2012 – Section 15)

N. EFFECTS OF WEAK-FIELD INTERACTIONS ON NON-LINEAR BIOLOGICAL OSCILLATORS AND SYNCHRONIZED NEURAL ACTIVITY

A unifying hypothesis for a plausible biological mechanism to account for very weak field EMF bioeffects other than cancer may lie with weak field interactions of pulsed RFR and ELF-modulated RFR as disrupters of synchronized neural activity. Electrical rhythms in our brains can be influenced by external signals. This is consistent with established weak field effects on coupled biological oscillators in living tissues. Biological systems of the heart, brain and gut are dependent on the cooperative actions of cells that function according to principles of non-linear, coupled biological oscillations for their synchrony, and are dependent on exquisitely timed cues from the environment at vanishingly small levels (Buzsaki, 2006; Strogatz, 2003). The key to synchronization is the joint actions of cells that co-operate electrically - linking populations of biological oscillators that couple together in large arrays and synchronize spontaneously. Synchronous biological oscillations in cells (pacemaker cells) can be disrupted by artificial, exogenous environmental signals, resulting in desynchronization of neural activity that regulates critical functions (including metabolism) in the brain, gut and heart and circadian rhythms governing sleep and hormone cycles (Strogatz, 1987). The brain contains a population of oscillators with distributed natural frequencies, which pull one another into synchrony (the circadian pacemaker cells). Strogatz has addressed the unifying mathematics of biological cycles and external factors disrupt these cycles (Strogatz, 2001, 2003). *"Rhythms can be altered by a wide variety of agents and that these perturbations must seriously alter brain performance"* (Buzsaki, 2006).

"Organisms are biochemically dynamic. They are continuously subjected to time-varying conditions in the form of both extrinsic driving from the environment and intrinsic rhythms generated by specialized cellular clocks within the organism itself. Relevant examples of the latter are the cardiac pacemaker located at the sinoatrial node in mammalian hearts (1) and the circadian clock residing at the suprachiasmatic nuclei in mammalian brains (2). These rhythm generators are composed of thousands of clock cells that are intrinsically diverse but nevertheless manage to function in a coherent oscillatory state. This is the case, for instance, of the circadian oscillations exhibited by the suprachiasmatic nuclei, the period of which is known to be determined by the mean period of the individual neurons making up the circadian clock (3–7). The mechanisms by which this collective behavior arises remain to be understood." (Strogatz, 2001; Strogatz, 2003)

Synchronous biological oscillations in cells (pacemaker cells) can be disrupted by artificial, exogenous environmental signals, resulting in desynchronization of neural activity that regulates critical functions (including metabolism) in the brain, gut and heart and circadian rhythms governing sleep and hormone cycles. The brain contains a population of oscillators with distributed natural frequencies, which pull one another into synchrony (the circadian pacemaker cells). Strogatz has addressed the unifying mathematics of biological cycles and external factors disrupt these cycles.

EMF AND RFR MAKE CHEMICAL TOXINS MORE HARMFUL

EMF acts on the body like other environmental toxicants do (heavy metals, organic chemicals and pesticides). Both toxic chemicals and EMF may generate free radicals, produce stress proteins and cause indirect damage to DNA. Where there is combined exposure the damages may add or even synergistically interact, and result in worse damage to genes.
(Sage and Carpenter, 2012 – Section 24)

EMF IS SUCCESSFULLY USED IN HEALING AND DISEASE TREATMENTS

"The potential application of the up-regulation of the HSP70 gene by both ELF-EMF and nanosecond PEMF in clinical practice would include trauma, surgery, peripheral nerve damage, orthopedic fracture, and vascular graft support, among others. Regardless of pulse design, EMF technology has been shown to be effective in bone healing [5], wound repair [11] and neural regeneration [31,36,48,49,51,63,64,65,66]. In terms of clinical applica- tion, EMF-induction of elevated levels of hsp70 protein also confers protection against hypoxia [61] and aid myocardial function and survival [20,22]. Given these results, we are particularly interested in the translational significance of effect vs. efficacy which is not usually reported by designers or investigators of EMF devices. More precise description of EM pulse and sine wave parameters, including the specific EM output sector, will provide consistency and "scientific basis" in reporting findings."

"The degree of electromagnetic field-effects on biological systems is known to be dependent on a number of criteria in the waveform pattern of the exposure system used; these include frequency, duration, wave shape, and relative orientation of the fields [6,29,32,33,39,40]. In some cases pulsed fields have demonstrated increased efficacy over static designs [19,21] in both medical and experimental settings." (Madkan et al, 2009)

(Sage and Carpenter, 2012 – Section 24)

ELF-EMF AND RFR ARE CLASSIFIED AS POSSIBLE CANCER-CAUSING AGENTS – WHY ARE GOVERNMENTS NOT ACTING?

The World Health Organization International Agency for Research on Cancer has classified wireless radiofrequency as a Possible Human Carcinogen (May, 2011)*. The designation applies to low-intensity RFR in general, covering all RFR-emitting devices and exposure sources (cell and cordless phones, WI-FI, wireless laptops, wireless hotspots, electronic baby monitors, wireless classroom access points, wireless antenna facilities, etc). The IARC Panel could have chosen to classify RFR as a Group 4 – Not A Carcinogen if the evidence was clear that RFR is not a cancer-causing agent. It could also have found a Group 3 designation was a good interim choice (Insufficient Evidence). IARC did neither.

(Sage and Carpenter, 2012 – Section 24)

NEW SAFETY LIMITS MUST BE ESTABLISHED - HEALTH AGENCIES SHOULD ACT NOW

Existing public safety limits (FCC and ICNIRP public safety limits) do not sufficiently protect public health against chronic exposure from very low-intensity exposures. If no mid-course corrections are made to existing and outdated safety limits, such delay will magnify the public health impacts with even more applications of wireless-enabled technologies exposing even greater populations around the world in daily life. (Sage and Carpenter, 2012 – Section 24)

SCIENTIFIC BENCHMARKS FOR HARM PLUS SAFETY MARGIN = NEW SAFETY LIMITS THAT ARE VALID

Health agencies and regulatory agencies that set public safety standards for ELF-EMF and RFR should act now to adopt new, biologically-relevant safety limits that key to the lowest scientific benchmarks for harm coming from the recent studies, plus a lower safety margin. Existing public safety limits are too high by several orders of magnitude, if prevention of bioeffects and minimization or elimination of resulting adverse human health effects. Most safety standards are a thousand times or more too high to protect healthy populations, and even less effective in protecting sensitive subpopulations.

(Sage and Carpenter, 2012 – Section 24)

SENSITIVE POPULATIONS MUST BE PROTECTED

Safety standards for sensitive populations will more likely need to be set at lower levels than for healthy adult populations. Sensitive populations include the developing fetus, the infant, children, the elderly, those with pre-existing chronic diseases, and those with developed electrical sensitivity (EHS). (Sage and Carpenter, 2012 – Section 24)

PROTECTING NEW LIFE - INFANTS AND CHILDREN

Strong precautionary action and clear public health warnings are warranted immediately to help prevent a global epidemic of brain tumors resulting from the use of wireless devices (mobile phones and cordless phones). Common sense measures to limit both ELF-EMF and RFR in the fetus and newborn infant (sensitive populations) are needed, especially with respect to avoidable exposures like baby monitors in the crib and baby isolettes (incubators) in hospitals that can be modified; and where education of the pregnant mother with respect to laptop computers, mobile phones and other sources of ELF-EMF and RFR are easily instituted.

(Sage and Carpenter, 2012 – Section 24)

Wireless laptops and other wireless devices should be strongly discouraged in schools for children of all ages. (Sage and Carpenter, 2012 – Section 24)

STANDARD OF EVIDENCE FOR JUDGING THE SCIENCE

The standard of evidence for judging the scientific evidence should be based on good public health principles rather than demanding scientific certainty before actions are taken. (Sage and Carpenter, 2012 – Section 24)

WIRELESS WARNINGS FOR ALL

The continued rollout of wireless technologies and devices puts global public health at risk from unrestricted wireless commerce unless new, and far lower exposure limits and strong precautionary warnings for their use are implemented.

(Sage and Carpenter, 2012 – Section 24)

EMF AND RFR ARE PREVENTABLE TOXIC EXPOSURES

We have the knowledge and means to save global populations from multi-generational adverse health consequences by reducing both ELF and RFR exposures. Proactive and immediate measures to reduce unnecessary EMF exposures will lower disease burden and rates of premature death.

(Sage and Carpenter, 2012 – Section 24)

DEFINING A NEW ‘EFFECT LEVEL’ FOR RFR

On a precautionary public health basis, a reduction from the BioInitiative 2007 recommendation of 0.1 uW/cm2 (or one-tenth of a microwatt per square centimeter) for cumulative outdoor RFR down to something three orders of magnitude lower (in the low nanowatt per square centimeter range) is justified.

A scientific benchmark of 0.003 uW/cm2 or three nanowatts per centimeter squared for ‘lowest observed effect level’ for RFR is based on mobile phone base station-level studies. Applying a ten-fold reduction to compensate for the lack of long-term exposure (to provide a safety buffer for chronic exposure, if needed) or for children as a sensitive subpopulation yields a 300 to 600 picowatts per square centimeter precautionary action level. This equates to a 0.3 nanowatts to 0.6 nanowatts per square centimeter as a reasonable, precautionary action level for chronic exposure to pulsed RFR.

These levels may need to change in the future, as new and better studies are completed. We leave room for future studies that may lower or raise today’s observed ‘effects levels’ and should be prepared to accept new information as a guide for new precautionary actions.

(Sage and Carpenter, 2012 – Section 24)

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure

(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

Power Density (Microwatts/centimeter2 - uW/cm2)		**Reference**
As low as (10^{-13}) or 100 femtowatts/cm2	Super-low intensity RFR effects at MW reasonant frequencies resulted in changes in genes; problems with chromatin conformation (DNA)	Belyaev, 1997
5 picowatts/cm2 (10^{-12})	Changed growth rates in yeast cells	Grundler, 1992
0.1 nanowatt/cm2 (10^{-10}) or 100 picowatts/cm2	Super-low intensity RFR effects at MW reasonant frequencies resulted in changes in genes; problems with chromatin condensation (DNA) intensities comparable to base stations	Belyaev, 1997
0.00034 uW/cm2	Chronic exposure to mobile phone pulsed RF significantly reduced sperm count,	Behari, 2006
0.0005 uW/cm2	RFR decreased cell proliferation at 960 MHz GSM 217 Hz for 30-min exposure	Velizarov, 1999
0.0006 - 0.0128 uW/cm2	Fatigue, depressive tendency, sleeping disorders, concentration difficulties, cardio- vascular problems reported with exposure to GSM 900/1800 MHz cell phone signal at base station level exposures.	Oberfeld, 2004
0.003 - 0.02 uW/cm2	In children and adolescents (8-17 yrs) short-term exposure caused headache, irritation, concentration difficulties in school.	Heinrich, 2010
0.003 to 0.05 uW/cm2	In children and adolescents (8-17 yrs) short-term exposure caused conduct problems in school (behavioral problems)	Thomas, 2010
0.005 uW/cm2	In adults (30-60 yrs) chronic exposure caused sleep disturbances, (but not significantly increased across the entire population)	Mohler, 2010
0.005 - 0.04 uW/cm2	Adults exposed to short-term cell phone radiation reported headaches, concentration difficulties (differences not significant, but elevated)	Thomas, 2008
0.006 - 0.01 uW/cm2	Chronic exposure to base station RF (whole-body) in humans showed increased stress hormones; dopamine levels substantially decreased; higher levels of adrenaline and nor-adrenaline; dose-response seen; produced chronic physiological stress in cells even after 1.5 years.	Buchner, 2012
0.01 - 0.11 uW/cm2	RFR from cell towers caused fatigue, headaches, sleeping problems	Navarro, 2003

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effects	Sleep, neuron firing rate, EEG, memory, learning, behavior
Oxidative damage/ROS/DNA damage/DNA repair failure	Cancer (other than brain), cell proliferation
Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure

(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

Power Density (Microwatts/centimeter2 - uW/cm2)		Reference
0.01 - 0.05 uW/cm2	Adults (18-91 yrs) with short-term exposure to GSM cell phone radiation reported headache, neurological problems, sleep and concentration problems.	Hutter, 2006
0.005 - 0.04 uW/cm2	Adults exposed to short-term cell phone radiation reported headaches, concentration difficulties (differences not significant, but elevated)	Thomas, 2008
0.015 - 0.21 uW/cm2	Adults exposed to short-term GSM 900 radiation reported changes in mental state (e.g., calmness) but limitations of study on language descriptors prevented refined word choices (stupified, zoned-out)	Augner, 2009
0.05 - 0.1 uW/cm2	RFR linked to adverse neurological, cardio symptoms and cancer risk	Khurana, 2010
0.05 - 0.1 uW/cm2	RFR related to headache, concentration and sleeping problems, fatigue	Kundi, 2009
0.07 - 0.1 uW/cm2	Sperm head abnormalities in mice exposed for 6-months to base station level RF/MW. Sperm head abnormalities occurred in 39% to 46% exposed mice (only 2% in controls) abnormalities was also found to be dose dependent. The implications of the pin-head and banana-shaped sperm head. The occurrence of sperm head observed increase occurrence of sperm head abnormalities on the reproductive health of humans living in close proximity to GSM base stations were discussed."	Otitoloju, 2010
0.38 uW/cm2	RFR affected calcium metabolism in heart cells	Schwartz, 1990
0.8 - 10 uW/cm2	RFR caused emotional behavior changes, free-radical damage by super-weak MWs	Akoev, 2002
0.13 uW/cm2	RFR from 3G cell towers decreased cognition, well-being	Zwamborn, 2003
0.16 uW/cm2	Motor function, memory and attention of school children affected (Latvia)	Kolodynski, 1996
0.168 - 1.053 uW/cm2	Irreversible infertility in mice after 5 generations of exposure to RFR from an 'antenna park'	Magras & Zenos, 1997
0.2 - 8 uW/cm2	RFR caused a two-fold increase in leukemia in children	Hocking, 1996
0.2 - 8 uW/cm2	RFR decreased survival in children with leukemia	Hocking, 2000
0.21 - 1.28 uW/cm2	Adolescents and adults exposed only 45 min to UMTS cell phone radiation reported increases In headaches.	Riddervold, 2008

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effects	Sleep, neuron firing rate, EEG, memory, learning, behavior
Oxidative damage/ROS/DNA damage/DNA repair failure	Cancer (other than brain), cell proliferation
Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure

(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

Power Density (Microwatts/centimeter2 - uW/cm2)		**Reference**
0.5 uW/cm2	Significant degeneration of seminiferous epithelium in mice at 2.45 GHz, 30-40 min.	Saunders, 1981
0.5 - 1.0 uW/cm2	Wi-FI level laptop exposure for 4-hr resulted in decrease in sperm viability, DNA fragmentation with sperm samples placed in petri dishes under a laptop connected via WI-FI to the internet.	Avendano, 2012
1.0 uW/cm2	RFR induced pathological leakage of the blood-brain barrier	Persson, 1997
1.0 uW/cm2	RFR caused significant effect on immune function in mice	Fesenko, 1999
1.0 uW/cm2	RFR affected function of the immune system	Novoselova, 1999
1.0 uW/cm2	Short-term (50 min) exposure in electrosensitive patients, caused loss of well-being after GSM and especially UMTS cell phone radiation exposure	Eltiti, 2007
1.3 - 5.7 uW/cm2	RFR associated with a doubling of leukemia in adults	Dolk, 1997
1.25 uW/cm2	RFR exposure affected kidney development in rats (in-utero exposure)	Pyrpasopoulou, 2004
1.5 uW/cm2	RFR reduced memory function in rats	Nittby, 2007
2 uW/cm2	RFR induced double-strand DNA damage in rat brain cells	Kesari, 2008
2.5 uW/cm2	RFR affected calcium concentrations in heart muscle cells	Wolke, 1996
2 - 4 uW/cm2	Altered cell membranes; acetycholine-induced ion channel disruption	D'Inzeo, 1988
4 uW/cm2	RFR caused changes in hippocampus (brain memory and learning)	Tattersall, 2001
4 - 15 uW/cm2	Memory impairment, slowed motor skills and retarded learning in children	Chiang, 1989
5 uW/cm2	RFR caused drop in NK lymphocytes (immune function decreased)	Boscolo, 2001
5.25 uW/cm2	20 minutes of RFR at cell tower frequencies induced cell stress response	Kwee, 2001
5 - 10 uW/cm2	RFR caused impaired nervous system activity	Dumansky, 1974
6 uW/cm2	RFR induced DNA damage in cells	Phillips, 1998

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effects	Sleep, neuron firing rate, EEG, memory, learning, behavior
Oxidative damage/ROS/DNA damage/DNA repair failure	Cancer (other than brain), cell proliferation
Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure

(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

Power Density (Microwatts/centimeter2 - uW/cm2)		Reference
8.75 uW/cm2	RFR at 900 MHz for 2-12 hours caused DNA breaks in leukemia cells	Marinelli, 2004
10 uW/cm2	Changes in behavior (avoidance) after 0.5 hour exposure to pulsed RFR	Navakatikian, 1994
10 - 100 uW/cm2	Increased risk in radar operators of cancer; very short latency period; dose response to exposure level of RFR reported.	Richter, 2000
12.5 uW/cm2	RFR caused calcium efflux in cells - can affect many critical cell functions	Dutta, 1989
13.5 uW/cm2	RFR affected human lymphocytes - induced stress response in cells	Sarimov, 2004
20 uW/cm2	Increase in serum cortisol (a stress hormone)	Mann, 1998
28.2 uW/cm2	RFR increased free radical production in rat cells	Yurekli, 2006
37.5 uW/cm2	Immune system effects - elevation of PFC count (antibody producing cells	Veyret, 1991
45 uW/cm2	Pulsed RFR affected serum testosterone levels in mice	Forgacs, 2006
50 uW/cm2	Cell phone RFR caused a pathological leakage of the blood-brain barrier in 1 hour	Salford, 2003
50 uW/cm2	An 18% reduction in REM sleep (important to memory and learning functions)	Mann, 1996
60 uW/cm2	RFR caused structural changes in cells of mouse embryos	Somozy, 1991
60 uW/cm2	Pulsed RFR affected immune function in white blood cells	Stankiewicz, 2006
60 uW/cm2	Cortex of the brain was activated by 15 minutes of 902 MHz cell phone	Lebedeva, 2000
65 uW/cm2	RFR affected genes related to cancer	Ivaschuk, 1999
92.5 uW/cm2	RFR caused genetic changes in human white blood cells	Belyaev, 2005
100 uW/cm2	Changes in immune function	Elekes, 1996
100 uW/cm2	A 24.3% drop in testosterone after 6 hours of CW RFR exposure	Navakatikian, 1994
120 uW/cm2	A pathological leakage in the blood-brain barrier with 915 MHz cell RF	Salford, 1994

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effects	Sleep, neuron firing rate, EEG, memory, learning, behavior
Oxidative damage/ROS/DNA damage/DNA repair failure	Cancer (other than brain), cell proliferation
Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

Power Density (Microwatts/centimeter2 - uW/cm2)		Reference
500 uW/cm2	Intestinal epithelial cells exposed to 2.45 GHz pulsed at 16 Hz showed changes in intercellular calcium.	Somozy, 1993
500 uW/cm2	A 24.6% drop in testosterone and 23.2% drop in insulin after 12 hrs of pulsed RFR exposure.	Navakatikian, 1994

STANDARDS		
530 - 600 uW/cm2	Limit for uncontrolled public exposure to 800-900 MHz	ANSI/IEEE and FCC
1000 uW/cm2	PCS STANDARD for public exposure (as of September 1,1997)	FCC, 1996
5000 uW/cm2	PCS STANDARD for occupational exposure (as of September 1, 1997)	FCC, 1996
BACKGROUND LEVELS		
0.003 uW/cm2	Background RF levels in US cities and suburbs in the 1990s	Mantiply, 1997
0.05 uW/cm2	Median ambient power density in cities in Sweden (30-2000 MHz)	Hamnierius, 2000
0.1 - 10 uW/cm2	Ambient power density within 100-200' of cell site in US (data from 2000)	Sage, 2000

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effects	Sleep, neuron firing rate, EEG, memory, learning, behavior
Oxidative damage/ROS/DNA damage/DNA repair failure	Cancer (other than brain), cell proliferation
Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure

(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

SAR (Watts/Kilogram)		Reference
0.000064 - 0.000078 W/Kg	Well-being and cognitive function affected in humans exposed to GSM-UMTS cell phone frequencies; RF levels similar near cell sites	TNO Physics and
0.00015 - 0.003 W/Kg	Calcium ion movement in isolated frog heart tissue is increased 18% (P<.01) and by 21% (P<.05) by weak RF field modulated at 16 Hz	Schwartz, 1990
0.000021 - 0.0021 W/Kg	Changes in cell cycle; cell proliferation (960 MHz GSM mobile phone)	Kwee, 1997
0.0003 - 0.06 W/Kg	Neurobehavioral disorders in offspring of pregnant mice exposed in utero to cell phones - dose-response impaired glutamatergic synaptic transmission onto layer V pyramidal neurons of the prefrontal cortex. Hyperactivity and impaired memory function in offspring. Altered brain development.	Aldad, 2012
0.0016 - 0.0044 W/Kg	Very low power 700 MHz CW affects excitability of hippocampus tissue, consistent with reported behavioral changes.	Tattersall, 2001
0.0021 W/Kg	Heat shock protein HSP 70 is activated by very low intensity microwave exposure in human epithelial amnion cells	Kwee, 2001
0.0024 - 0.024 W/Kg	Digital cell phone RFR at very low intensities causes DNA damage in human cells; both DNA damage and impairment of DNA is reported	Phillips, 1998
0.0027 W/Kg	Changes in active avoidance conditioned behavioral effect is seen after one-half hour of pulsed radiofrequency radiation	Navakatikian, 1994
0.0035 W/Kg	900 MHz cell phone signal induces DNA breaks and early activation of p53 gene; short exposure of 2-12 hours leads cells to acquire greater survival chance - linked to tumor agressiveness.	Marinelli, 2004
0.0095 W/Kg	MW modulated at 7 Hz produces more errors in short-term memory functioin on complex tasks (can affect cognitive processes such as attention and memory)	Lass, 2002
0.001 W/Kg	750 MHz continuous wave (CW) RFR exposure caused increase in heat shock protein (stress proteins). Equivalent to what would be induced by 3 degree C. heating of tissue (but no heating occurred)	De Pomerai, 2000
0.001 W/Kg	Statistically significant change in intracellular calcium concentration in heart muscle cells exposed to RFR (900 MHz/50 Hz modulation)	Wolke, 1996

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effects	Sleep, neuron firing rate, EEG, memory, learning, behavior
Oxidative damage/ROS/DNA damage/DNA repair failure	Cancer (other than brain), cell proliferation
Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure
(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

SAR (Watts/Kilogram)		**Reference**
0.0021 W/Kg	A significant change in cell proliferation not attributable to thermal heating. RFR induces non-thermal stress proteins (960 MHz GSM)	Velizarov, 1999
0.004 - 0.008 W/Kg	915 MHz cell phone RFR caused pathological leakage of blood-brain barrier. Worst at lower SAR levels and worse with CW compared to Frequency of pathological changes was 35% in rats exposed to pulsed radiation at 50% to continuous wave RFR. Effects observed at a specific absorption (SA) of > 1.5 joules/Kg in human tissues	Persson, 1997
0.0059 W/Kg	Cell phone RFR induces glioma (brain cancer) cells to significantly increase thymidine uptake, which may be indication of more cell division	Stagg, 1997
0.014 W/Kg	Sperm damage from oxidative stress and lowered melatonin levels resulted from 2-hr per day/45 days exposure to 10 GHz.	Kumar, 2012
0.015 W/Kg	Immune system effects - elevation of PFC count (antibody-producing cells)	Veyret, 1991
0.02 W/Kg	A single, 2-hr exposure to GSM cell phone radiation results in serious neuron damage (brain cell damage) and death in cortex, hippocampus, and basal ganglia of brain- even 50+ days later blood-brain barrier is still leaking albumin (P<.002) following only one cell phone exposure	Salford, 2003
0.026 W/Kg	Activity of c-jun (oncogene or cancer gene) was altered in cells after 20 minutes exposure to cell phone digital TDMA signal	Ivaschuk, 1997
0.0317 W/Kg	Decrease in eating and drinking behavior	Ray, 1990
0.037 W/Kg	Hyperactivity caused by nitric oxide synthase inhibitor is countered by exposure to ultra-wide band pulses (600/sec) for 30 min	Seaman, 1999
0.037 - 0.040 W/Kg	A 1-hr cell phone exposure causes chromatin condensation; impaired DNA repair mechanisms; last 3 days (longer than stress response) the effect reaches saturation in only one hour of exposure; electro- sensitive (ES) people have different response in formation of DNA repair foci, compared to healthy individuals; effects depend on carrier frequency (915 MHz = 0.037 W/Kg but 1947 MHz = 0.040 W/Kg)	Belyaev, 2008
0.05 W/Kg	Significant increase in firing rate of neurons (350%) with pulsed 900 MHz cell phone radiation exposure (but not with CW) in avian brain cells	Beason, 2002

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
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Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure

(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

SAR (Watts/Kilogram)		Reference
0.09 W/Kg	900 MHz study of mice for 7 days, 12-hr per day (whole-body) resulted in significant effect on mitochondria and genome stability	Aitken, 2005
0.091 W/Kg	Wireless internet 2400 MHz, 24-hrs per day/20 weeks increased DNA damage and reduced DNA repair; levels below 802.11 g Authors say "findings raise questions about safety of radiofrequency exposure from Wi-Fi internet access devices for growing organisms of reproductive age, with a potential effect on fertility and integrity of germ cells" (male germ cells are the reproductive cells=sperm)	Atasoy, 2012
0.11 W/Kg	Increased cell death (apoptosis) and DNA fragmentation at 2.45 GHz for 35 days exposure (chronic exposure study)	Kesari, 2010
0.121 W/Kg	Cardiovascular system shows significant decrease in arterial blood pressure (hypotension) after exposure to ultra-wide band pulses	Lu, 1999
0.13 - 1.4 W/Kg	Lymphoma cancer rate doubled with two 1/2-hr exposures per day of cell phone radiation for 18 months (pulsed 900 MHz cell signal)	Repacholi, 1997
0.14 W/Kg	Elevation of immune response to RFR exposure	Elekes, 1996
0.141 W/Kg	Structural changes in testes - smaller diameter of seminiferous	Dasdag, 1999
0.15 - 0.4 W/Kg	Statistically significant increase in malignant tumors in rats chronically exposed to RFR	Chou, 1992
0.26 W/Kg	Harmful effects to the eye/certain drugs sensitize the eye to RFR	Kues, 1992
0.28 - 1.33 W/Kg	Significant increase in reported headaches with increasing use of hand-held cell phone use (maximum tested was 60 min per day)	Chia, 2000
0.3 - 0.44 W/Kg	Cell phone use results in changes in cognitive thinking/mental tasks related to memory retrieval	Krause, 2000
0.3 - 0.44 W/Kg	Attention function of brain and brain responses are speeded up	Preece, 1999
0.3 - 0.46 W/Kg	Cell phone RFR doubles pathological leakage of blood-brain barrier permeability at two days (P=.002) and triples permeability at four days (P=.001) at 1800 MHz GSM cell phone radiation	Schirmacher, 2000
0.43 W/Kg	Significant decrease in sperm mobility; drop in sperm concentration; and decrease in seminiferous tubules at 800 MHz, 8-hr/day, 12 weeks, with mobile phone radiation level on STANDBY ONLY (in rabbits)	Salama, 2008

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
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Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure

(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

SAR (Watts/Kilogram)		**Reference**
0.5 W/Kg	900 MHz pulsed RF affects firing rate of neurons (Lymnea stagnalis) but continuous wave had no effect	Bolshakov, 1992
0.58 - 0.75 W/Kg	Decrease in brain tumors after chronic exposure to RFR at 836 MHz	Adey, 1999
0.6 - 0.9 W/Kg	Mouse embryos develop fragile cranial bones from in utero 900 MHz The authors say "(O)ur results clearly show that even modest exposure (e.g., 6 min daily for 21 days" is sufficient to interfere with the normal mouse developmental process"	Fragopoulou, 2009
0.6 and 1.2 W/Kg	Increase in DNA single and double-strand DNA breaks in rat brain cells with exposure to 2450 MHz RFR	Lai & Singh, 1996
0.795 W/Kg	GSM 900 MHz, 217 Hz significantly decreases ovarian development and size of ovaries, due to DNA damage and premature cell death of nurse cells and follicles in ovaries (that nourish egg cells)	Panagopoulous, 2012
0.87 W/Kg	Altered human mental performance after exposure to GSM cell phone radiation (900 MHz TDMA digital cell phone signal)	Hamblin, 2004
0.87 W/Kg	Change in human brainwaves; decrease in EEG potential and statistically significant change in alpha (8-13 Hz) and beta (13-22 Hz) brainwave activity in humans at 900 MHz; exposures 6/min per day for 21 days (chronic exposure)	D'Costa, 2003
0.9 W/Kg	Decreased sperm count and more sperm cell death (apoptosis) after 35 days exposure, 2-hr per day	Kesari, 2012
< 1.0 W/Kg	Rats exposed to mobile phone radiation on STANDBY ONLY for 11-hr 45-min plus 15-min TRANSMIT mode; 2 times per day for 21 days showed decreased number of ovarian follicles in pups born to these pregnant rats. The authors conclude "the decreased number of follicles in pups exposed to mobile phone microwaves suggest that intrauterine exposure has toxic effects on ovaries."	Gul, 2009
0.4 - 1.0 W/Kg	One 6-hr exposure to 1800 MHz cell phone radiation in human sperm cells caused a significant dose response and reduced sperm motility and viability; reactive oxygen species levels were significantly increased after exposure to 1.0 W/Kg; study confirms detrimental effects of RF/MW to human sperm. The authors conclude "(T)hese findings have clear implicatiions for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring."	De Iuliis, 2009
1.0 W/Kg	Human semen degraded by exposure to cell phone frequency RF increased free-radical damage.	De Iuliis, 2009

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
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(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

SAR (Watts/Kilogram)		Reference
1.0 W/Kg	Motility, sperm count, sperm morphology, and viability reduced in active cell phone users (human males) in dose-dependent manner.	Agarwal, 2008
1.0 W/Kg	GSM cell phone use modulates brain wave oscillations and sleep EEG	Huber, 2002
1.0 W/Kg	Cell phone RFR during waking hours affects brain wave activity. (EEG patterns) during subsequent sleep	Achermann, 2000
1.0 W/Kg	Cell phone use causes nitric oxide (NO) nasal vasodilation (swelling inside nasal passage) on side of head phone use	Paredi, 2001
1.0 W/Kg	Increase in headache, fatigue and heating behind ear in cell phone users	Sandstrom, 2001
1.0 W/Kg	Significant increase in concentration difficulties using 1800 MHz cell phone compared to 900 MHz cell phone	Santini, 2001
1.0 W/Kg	Sleep patterns and brain wave activity are changed with 900 MHz cell phone radiation exposure during sleep	Borbely, 1999
1.4 W/Kg	GSM cell phone exposure induced heat shock protein HSP 70 by 360% (stress response) and phosphorylation of ELK-1 by 390%	Weisbrot, 2003
1.46 W/Kg	850 MHz cell phone radiation decreases sperm motility, viability is significantly decreased; increased oxidative damage (free-radicals) significantly decreased; increased oxidative damage (free-radicals)	Agarwal, 2009
1.48 W/Kg	A significant decrease in protein kinase C activity at 112 MHz with 2-hr per day for 35 days; hippocampus is site, consistent with reports that RFR negatively affects learning and memory functions	Paulraj, 2004
1.0 - 2.0 W/Kg	Significant elevation in micronuclei in peripheral blood cells at 2450 MHz (8 treatments of 2-hr each)	Trosic, 2002
1.5 W/Kg	GSM cell phone exposure affected gene expression levels in tumor suppressor p53-deficient embryonic stem cells; and significantly increased HSP 70 heat shock protein production	Czyz, 2004
1.8 W/Kg	Whole-body exposure to RF cell phone radiation of 900-1800 MHz 1 cm from head of rats caused high incidence of sperm cell death; deformation of sperm cells; prominent clumping together of sperm cells into "grass bundle shapes" that are unable to separate/swim. Sperm cells unable to swim and fertilize in normal manner.	Yan, 2007

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effects	Sleep, neuron firing rate, EEG, memory, learning, behavior
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Reported Biological Effects from Radiofrequency Radiation at Low-Intensity Exposure

(Cell Tower, Wi-Fi, Wireless Laptop and 'Smart' Meter RF Intensities)

SAR (Watts/Kilogram)		**Reference**
2.0 W/Kg	GSM cell phone exposure of 1-hr activated heat shock protein HSP 27 (stress response) and P38 MAPK (mutagen-activated protein kinase) that authors say facilitates brain cancer and increased blood-brain barrier permeability, allowing toxins to cross BBB into brain	Leszczynski, 2002
2 W/Kg	900 MHz cell phone exposure caused brain cell oxidative damage by increasing levels of NO, MDA, XO and ADA in brain cells; caused statistically significant increase in 'dark neurons' or damaged brain cells in cortex, hippocampus and basal ganglia with a 1-hr exposure for 7 consecutive days	Ilhan, 2004
2.6 W/Kg	900 MHz cell phone exposure for 1-hr significantly altered protein expression levels in 38 proteins following irradiation; activates P38 MAP kinase stress signalling pathway and leads to changes in cell sie and shape (shrinking and rounding up) and to activation of HSP 27, a stress protein (heat shock protein)	Leszczynski, 2004
2.0 - 3.0 W/Kg	RFR accelerated development of both skin and breast tumors	Szmigielski, 1982
2 W/Kg	Pulse-modulated RFR and MF affect brain physiology (sleep study)	Schmidt, 2012

STANDARDS		
0.08 W/Kg	IEEE Standard uncontrolled public environment (whole body)	IEEE
0.4 W/Kg	IEEE Standard controlled occupational environment (whole body)	IEEE
1.6 W/Kg	FCC (IEEE) SAR limit for 1 gram of tissue in a partial body exposure	FCC, 1996
2 W/Kg	ICNIRP SAR limit for 10 grams of tissue	ICNIRP, 1996

Stress proteins, HSP, disrupted immune function	Brain tumors and blood-brain barrier
Reproduction/fertility effects	Sleep, neuron firing rate, EEG, memory, learning, behavior
Oxidative damage/ROS/DNA damage/DNA repair failure	Cancer (other than brain), cell proliferation
Disrupted calcium metabolism	Cardiac, heart muscle, blood-pressure, vascular effects

SECTION 2

Statement of the Problem

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Prepared for the BioInitiative Working Group

August 2007

STATEMENT OF THE PROBLEM

Background and Objectives

This Report is the product of an international research and public policy initiative to document what is known of biological effects that occur at low-intensity EMF exposures (for both radiofrequency radiation RF and power-frequency ELF, and various forms of combined exposures that are now known to be bioactive). The Report has been written to document the reasons why current public exposure standards for non-ionizing electromagnetic radiation are no longer good enough to protect public health.

A working group composed of scientists, researchers and public health policy professionals (The BioInitiative Working Group) has joined together to document the information that must be considered in the international debate about the adequacy (or inadequacy) of existing public exposure standards.

Recognizing that other bodies in the United States, United Kingdom, Australia, many European Union and eastern European countries as well as the World Health Organization are actively debating this topic, the BioInitiative Working Group has conducted a independent science and public health policy review process.

Objectives

1) To establish a working group
2) To evaluate literature reviews for IEEE (2006) and WHO (2007) initiatives on standards that have resulted in (or continue to recommend) no change in thermally-based public exposure limits.
3) To identify systematic screening-out techniques that consequently under-report, omit or overlook results of scientific studies reporting low-intensity bioeffects and/or potential health effects.
4) To document key scientific studies and reviews that identify low-intensity effects for which any new human exposure standards should provide safety limits.
5) To document key “chains of evidence” that must be taken into account in new human exposure standards (melatonin and free-radical production effects on DNA damage and/or repair; stress protein induction at low-intensity levels; etc.)
6) To write a rationale for a biologically-based human exposure standard,
7) To identify “next steps” in advancing biologically-based exposure standards that are protective of public health; that are derived in traditional public health approaches.

Eleven (11) chapters documenting key scientific studies and reviews that identify low-intensity effects of electromagnetic fields have been produced by the members of the BioInitiative Working Group; four additional chapters are provided that discuss public health considerations, how the scientific information should be evaluated in the context of prudent public health policy, and discussing the basis for taking precautionary and preventative actions that are proportionate to the knowledge at hand. Other scientific review bodies and agencies have reached different conclusions by adopting standards of evidence so unreasonably high as to exclude any finding of scientific concern, and thus justify retaining outdated thermal standards. The clear consensus of the BioInitiative Working Group members is that the existing public safety limits are inadequate. New approaches to development of public safety standards are needed based on biologically-based effects, rather than based solely on RF heating (or induced currents in the case of ELF). The Report concludes with recommended actions that are proportionate to the evidence and in accord with prudent public health policy.

The Report also presents information about what level of scientific evidence is sufficient to make changes now. It addresses the questions:

- What is "proof"? Do we need proof before we take any action? Is an unreasonably high and overly-restrictive definition of "proof" what is keeping some governments from facing the evidence that the need for new public exposure limits is demonstrated?
- What is sufficient evidence? How much evidence is needed? Do we have it yet?
- Do scientists and public health experts differ on when action is warranted? If so, how?
- What is the prudent course of action when the consequence of doing nothing is likely to have serious global consequences on public health, confidence in governments and social/economic resources?
- What are the costs of guessing wrong and under-reacting? Or, of over-reacting?
- Whose opinions should count in the process of deciding about health risks and harm?
- Is the global, governmental process addressing these questions transparent and responsive to public concerns? Or, is it a cosmetic process giving the illusion of transparency and democratic participation? Are some countries ostracized for views and actions that are more protective of public health? How can we equitably decide on the appropriate level of public protection within each country, when it is obvious that some countries would be best off spending their time and money on basic medical needs and infrastructure improvements to save lives, when others need to look at prevailing disease endpoints relevant to their populations, and wish to act accordingly?

- How has the effort for global harmonization of ELF and RF exposure standards thwarted the efforts of individual countries to read, reason and choose?
- How much control have special interests exerted over harmonization goals and safety standards? How much over scientific funding, research design, dissemination of research results and media control? Are the interests of the public being conserved?
- What actions are proportionate to the knowledge we now have? What is preventative action and how does it differ from precautionary action?

It describes what the existing exposure standards are, and how some international governmental bodies are standing by the old exposure standards despite evidence that change is needed.

A good way to compare what kind of actions should be taken now is to look at what has been done with other environmental toxicants. It is well-established that public health decision-makers should act before it is too late to prevent damage that can reasonably be expected now; especially where the harm may be serious and widespread. Some actions that can prevent future harm are identified. The basis for taking action now rather than later is explained. This report can serve as a basis for arguing the scientific and public health policy reasons that changes are needed. It documents information for decision-makers and the public who want to understand what is already known biological effects occuring at low-intensity exposures; and why it is reasonable to expect our governmental agencies to develop new, biologically-based exposure standards that protect the public.

Problems with Existing Public Health Standards (Safety Limits)

Today's public exposure limits are based on the presumption that heating is the only concern when living organisms are exposed to RF and ELF. These exposures can create tissue heating that is well known to be harmful in even very short-term doses. As such, thermal limits do serve a purpose. For example, for people whose occupations require them to work around electrical power lines or heat-sealers, or for people who install and service wireless antenna towers; thermally-based limits are necessary to prevent damage from heating (or, in the case of ELF - from induced currents in tissues). In the past, scientists and engineers developed exposure standards for electromagnetic radiation based what we now believe are faulty assumptions that the right way to measure how much non-ionizing energy humans can tolerate (how much exposure) without harm is to measure only the heating of tissue (for – induced currents in the body). In the last few decades, it has been established beyond any reasonable doubt that bioeffects and some adverse health effects occur at far lower levels of RF and exposure where no heating occurs at all; some effects are shown to occur at several hundred thousand times below the existing public safety limits where heating is an impossibility. Effects occur at non-thermal or low-intensity exposure levels far below the levels that federal agencies say should keep the public safe. For many new devices operating with wireless technologies, the devices are exempt from any regulatory standards. The existing standards have been proven to be inadequate to control against harm from low-intensity, chronic exposures, based on any reasonable, independent assessment of the scientific literature. It means that an entirely new basis (a biological basis) for new exposure standards is needed. New standards need to take into account what we have learned about the effects of non-ionizing electromagnetic fields and to design new limits based on biologically-demonstrated effects that are important to proper biological function in living organisms. It is vital to do so because the explosion of new sources has created unprecedented levels of artificial electromagnetic fields that now cover all but remote areas of the habitable space on earth. Mid-course corrections are needed in the way we accept, test and deploy new technologies that expose us to ELF and RF in order to avert public health problems of a global nature.

At least three decades of scientific study and observation of effects on humans and animals shows that non-thermal exposure levels can result in biologically-relevant effects. There should be no effects occurring at all. Yet, clearly they do occur. This means the standards for protecting public health are based on the wrong premise - that only what heats tissue can result in harm. It does appear that it is the INFORMATION conveyed by electromagnetic radiation, rather than the heat, which causes biological changes, some of which may lead to unwellness, illness and even death, According to Adey (2004):

> *"There are major unanswered questions about possible health risks that may arise from human exposures to various man-made electromagnetic fields where these exposures are intermittent, recurrent, and may extend over a significant portion of the lifetime of an individual. Current equilibrium thermodynamic models fail to explain an impressive spectrum of observed bioeffects at non-thermal exposure levels."*

Recent opinions by experts have documented deficiencies in current exposure standards. There is widespread discussion that thermal limits are outdated, and that biologically-based exposure standards are needed. Section 4 describes concerns expressed by WHO, 2007 in its Health Criteria Monograph; the SCENIHR Report, 2006 prepared for the European Commission; the UK SAGE Report, 2007; the Health Protection Agency, United Kingdom in 2005; the NATO Advanced Research Workshop in 2005; the US Radiofrequency Interagency Working Group in 1999; the US Food and Drug Administration in 2000 and 2007; the World Health Organization in 2002; the World Health Organization International Agency for Cancer Research (IARC, 2001), the United Kingdom Parliament Independent Expert Group Report (Stewart Report, 2000) and others.

A pioneer researcher, the late Dr. Ross Adey, in his last publication in Bioelectromagnetic Medicine (P. Roche and M. Markov, eds. 2004) concluded:

> "*There are major unanswered questions about possible health risks that may arise from exposures to various man-made electromagnetic fields where these human exposures are intermittent, recurrent, and may extend over a significant portion of the lifetime of the individual.*"[1]
>
> *"Epidemiological studies have evaluated and radiofrequency fields as possible risk factors for human health, with historical evidence relating rising risks of such factors as progressive rural electrification, and more recently, to methods of electrical power distribution and utilization in commercial buildings. Appropriate models describing these bioeffects are based in nonequilibrium thermodynamics, with nonlinear electrodynamics as an integral feature. Heating models, based in equilibrium thermodynamics, fail to explain an impressive new frontier of much greater significance. ..... Though incompletely understood, tissue free radical interactions with magnetic fields may extend to zero field levels. (Adey, 2004)*

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 3

The Existing Public Exposure Standards

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August 2007

The US Federal Communications Commission (FCC) Exposure Standard Recommendations

In the United States, the Federal Communications Commission (FCC) enforces limits for both occupational exposures (in the workplace) and public exposures. The exposure limits are variable according to the frequency (in megahertz) and the duration of exposure time (6 minutes for occupational and 30 minutes for public exposures). Table 3.1 show exposure limits for occupational and uncontrolled public access to radiofrequency radiation such as is emitted from AM, FM, television and wireless sources through the air. As an example, 583 microwatts/cm2 (µW/cm2) is the public limit for the 875 MHz cell phone wireless frequency and 1000 µW/cm2 is the limit for PCS frequencies in the 1800 – 1950 MHz range averaged over 30 minutes. The limits in Table 3.1 would pertain to exposures in the vicinity of transmitting antennas (not devices like cell phones, for which exposure limits are shown in Table 3.2).

The FCC is required by the National Environmental Policy Act of 1969 to evaluate the effect of emissions from FCC-regulated transmitters on the quality of the human environment. At the present time there is no federally-mandated radio frequency (RF) exposure standard. However, several non-government organizations, such as the American National Standards Institute (ANSI), the Institute of Electrical and Electronics Engineers, Inc. (IEEE), and the National Council on Radiation Protection and Measurements (NCRP) have issued recommendations for human exposure to RF electromagnetic fields. The FCC has endorsed these recommendations, and enforces compliance. http://www.fcc.gov/oet/rfsafety/

Table 3.1 FCC LIMITS FOR MAXIMUM PERMISSIBLE EXPOSURE (MPE)

(A) Limits for Occupational/Controlled Exposure

Frequency Range (MHz)	Electric Field Strength (E) (V/m)	Magnetic Field Strength (H) (A/m)	Power Density (S) (mW/cm2)	Averaging Time $[E]^2$ $[H]^2$ or S (minutes)
0.3-3.0	614	1.63	(100)*	6
3.0-30	1842/f	4.89/f	$(900/f_2)$*	6
30-300	61.4	0.163	1.0	6
300-1500			f/300	6
1500-100,000			5	6

(B) FCC Limits for General Population/Uncontrolled Exposure

Frequency Range (MHz)	Electric Field Strength (E) (V/m)	Magnetic Field Strength (H) (A/m)	Power Density (S) (mW/cm2)	Averaging Time $[E]^2$ $[H]^2$ or S (minutes)
0.3-3.0	614	1.63	(100)*	30
3.0-30	824/f	2.19/f	$(180/f_2)$*	30
30-300	27.5	0.073	0.2	30
300-1500	--	--	f/1500	30
1500-100,000	--	--	1.0	30

f = frequency in MHz

*Plane-wave equivalent power density

NOTE 1: ***Occupational/controlled*** limits apply in situations in which persons are exposed as a consequence of their employment provided those persons are fully aware of the potential for exposure and can exercise control over their exposure. Limits for occupational/controlled exposure also apply in situations when an individual is transient through a location where occupational/controlled limits apply provided he or she is made aware of the potential for exposure.

NOTE 2: ***General population/uncontrolled*** exposures apply in situations in which the general public may be exposed, or in which persons that are exposed as a consequence of their employment may not be fully aware of the potential for exposure or can not exercise control over their exposure.

Source: OET, 1997.

FCC Guidelines for Cell and PCS Phones (and other radiofrequency emitting devices)

Cell phones and portable transmitting devices that operate in the Cellular Radiotelephone Service, the Personal Communications Services (PCS), the Satellite Communications Services, the Maritime Services (ship earth stations only) and the Specialized Mobile Radio (SMR) Service are subject to routine environmental (not health) evaluation for RF exposure prior to equipment authorization or use by the FCC. Section 2.1093 of the FCC's Rules (47 CFR §2.1093) that apply to "portable" devices. For purposes of these requirements a portable device is defined as a transmitting device designed to be used so that the radiating structure(s) of the device is/are within 20 centimeters of the body of the user (OET, 1997).

Cell phones and some other wireless communication devices are regulated by the FCC according to their emissions, which depend on the amount of power absorbed into the body. The metric for measurement is specific absorption rate (SAR) and is expressed in watts per kilogram of tissue. The limit for absorption of radiofrequency radiation is limited to 1.6 W/kG within 1 gram of human tissue. This limit has been recommended for change (relaxation) by the IEEE in April of 2006. If adopted by the FCC, this amount of heat or 1.6 W/Kg would be measured over 10 times as much tissue (10 grams) so that far higher heating is possible from these devices over small amounts of tissue (would be far less strict that the current limit, if adopted). More cell phone and related PDA devices would then comply be able with the looser standard, and the public could potentially receive much higher radiofrequency radiation exposures, and it would be in compliance (legal).

“The SAR criteria to be used are specified below and apply for portable devices transmitting in the frequency range from 100 kHz to 6 GHz. The limits used for evaluation are based generally on criteria published by the Institute of Electrical and Electronics Engineers, Inc., (IEEE) for localized specific absorption rate ("SAR") in Section 4.2 of "IEEE Standard for Safety Levels with Respect to Human Exposure to Radio Frequency Electromagnetic Fields, 3 kHz to 300 GHz," ANSI/IEEE C95.1-1992.

These criteria for SAR evaluation are similar to those recommended by the National Council on Radiation Protection and Measurements (NCRP) in "Biological Effects and Exposure Criteria for Radiofrequency Electromagnetic Fields," NCRP Report No. 86, Section 17.4.5. Copyright NCRP, 1986, Bethesda, Maryland 20814.”

(1) FCC Limits for Occupational/Controlled exposure: 0.4 W/kg as averaged over the whole-body and spatial peak SAR not exceeding 8 W/kg as averaged over any 1 gram of tissue (defined as a tissue volume in the shape of a cube). Exceptions are the hands, wrists, feet and ankles where the spatial peak SAR shall not exceed 20 W/kg, as averaged over any 10 grams of tissue (defined as a tissue volume in the shape of a cube). Occupational/Controlled limits apply when persons are exposed as a consequence of their employment provided these persons are fully aware of and exercise control over their exposure. Awareness of exposure can be accomplished by use of warning labels or by specific training or education through appropriate means, such as an RF safety program in a work environment (OET, 1997).

(2) FCC Limits for General Population/Uncontrolled exposure: 0.08 W/kg as averaged over the whole-body and spatial peak SAR not exceeding 1.6 W/kg as averaged over any 1 gram of tissue (defined as a tissue volume in the shape of a cube). Exceptions are the hands, wrists, feet and ankles where the spatial peak SAR shall not exceed 4 W/kg, as averaged over any 10 grams of tissue (defined as a tissue volume in the shape of a cube). General Population/Uncontrolled limits apply when the general public may be exposed, or when persons that are exposed as a consequence of their employment may not be fully aware of the potential for exposure or do not exercise control over their exposure. Warning labels placed on consumer devices such as cellular telephones will not be sufficient reason to allow these devices to be evaluated subject to limits for occupational/controlled exposure (OET, 1997).

In the United States, two professional societies - the Institute of Electrical and Electronics Engineers, Inc. (IEEE) and the National Council for Radiation Protection and Measurements (NCRP) develop recommendations for safety standards. . The IEEE charter calls itself the world's leading professional association for the advancement of technology, as well as the instigator of public safety standards. The IEEE recommendations have historically been endorsed by the American National Standards Institute (ANSI) and finally considered by the FCC for implementation. The US Federal Communications Commission (FCC) may then take the recommendations and adopt them as mandatory exposure limits. Several standard-setting processes have occurred like this in the last few decades.

The most recent IEEE recommendations for 3 kHz to 300 GHz were developed in 2006 (IEEE, 2006). Rather than lower the existing limits for radiofrequency and microwave radiation exposure, they greatly increase the exposure limits. This is perplexing since it ignores or discounts a large body of scientific evidence clearly documenting biologically-relevant changes at levels LOWER (much lower) than the existing standards.

ICNIRP Guidelines (International Radiofrequency Guidelines)

In April 1998, the International Commission on Non-Ionizing Radiation Protection (ICNIRP) published guidelines for limiting exposure to time-varying electric, magnetic and electromagnetic fields in the frequency range up to 300 GHz.. These guidelines replaced previous advice issued in 1988 and 1990. The main objective of the ICNIRP Guidelines is to establish guidelines for limiting EMF exposure that will provide protection against known adverse health effects (ICNIRP, 1998). An adverse health effect is defined by ICNIRP as one which causes detectable impairment of the health of the exposed individual or of his or her offspring; a biological effect, on the other hand, may or may not result in an adverse health effect.

The guidelines presented in Table 3.2 apply to occupational and public exposure.

Table 3.2 ICNIRP Basic restrictions for time varying electric and magnetic fields for frequencies up to 10 GHz.

Exposure characteristics	Frequency range	Current density for head and trunk (mA m$^{!2}$)(rms)	Whole-body average SAR (W kg$^{!1}$)	Localized SAR (head and trunk) (W kg$^{!1}$)	Localized SAR (limbs) (W kg$^{!1}$)
Occupational exposure	up to 1 Hz	40	—	—	—
	1–4 Hz	40/*f*	—	—	—
	4 Hz–1 kHz	10	—	—	—
	1–100 kHz	*f*/100	—	—	—
	100 kHz–10 MHz	*f*/100	0.4	10	20
	10 MHz–10 GHz		0.4	10	20
General public exposure	up to 1 Hz	8	—	—	—
	1–4 Hz	8/*f*	—	—	—
	4 Hz–1 kHz	2	—	—	—
	1–100 kHz	*f*/500	—	—	—
	100 kHz–10 MHz	*f*/500	0.08	2	4
	10 MHz–10 GHz		0.08	2	4

Notes:

1. *f* is the frequency in hertz.
2. Because of electrical inhomogeneity of the body, current densities should be averaged over a cross-section of 1 cm^2 perpendicular to the current direction.
3. For frequencies up to 100 kHz, peak current density values can be obtained by multiplying the rms value by %2 (~1.414). For pulses of duration t_p the equivalent frequency to apply in the basic restrictions should be calculated as $f = 1/(2t_p)$. For frequencies up to 100 kHz and for pulsed magnetic fields, the maximum current density associated with the pulses can be calculated from the rise/fall times and the maximum rate of change of magnetic flux density. The induced current density can then be compared with the appropriate basic restriction.
4. All SAR values are to be averaged over any 6-minute period.
5. Localized SAR averaging mass is any 10 g of contiguous tissue; the maximum SAR so obtained should be the value used for the estimation of exposure.
6. For pulses of duration t_p the equivalent frequency to apply in the basic restrictions should be calculated as $f = 1/(2t_p)$. Additionally, for pulsed exposures, in the frequency range 0.3 to 10 GHz and for localized exposure of the head, in order to limit or avoid auditory effects caused by thermoelastic expansion, an additional basic restriction is recommended. This is that the SA should not exceed 10 mJ kg^{-1} for workers and 2 mJ kg^{-1} for the general public averaged over 10 g tissue.

In the frequency range from a few Hz to 1 kHz, for levels of induced current density above 100 mA m$^{!2}$, the thresholds for acute changes in central nervous system excitability and other acute effects such as reversal of the visually evoked potential are exceeded. In view of the safety considerations above, it was decided that, for frequencies in the range 4 Hz to 1 kHz, occupational exposure should be limited to fields that induce current densities less than 10 mA m$^{!2}$, i.e., to use a safety factor of 10. For the general public an additional factor of 5 is applied, giving a basic exposure restriction of 2 mA m$^{!2}$. Below 4 Hz and above 1 kHz, the basic restriction on induced current density increases progressively.

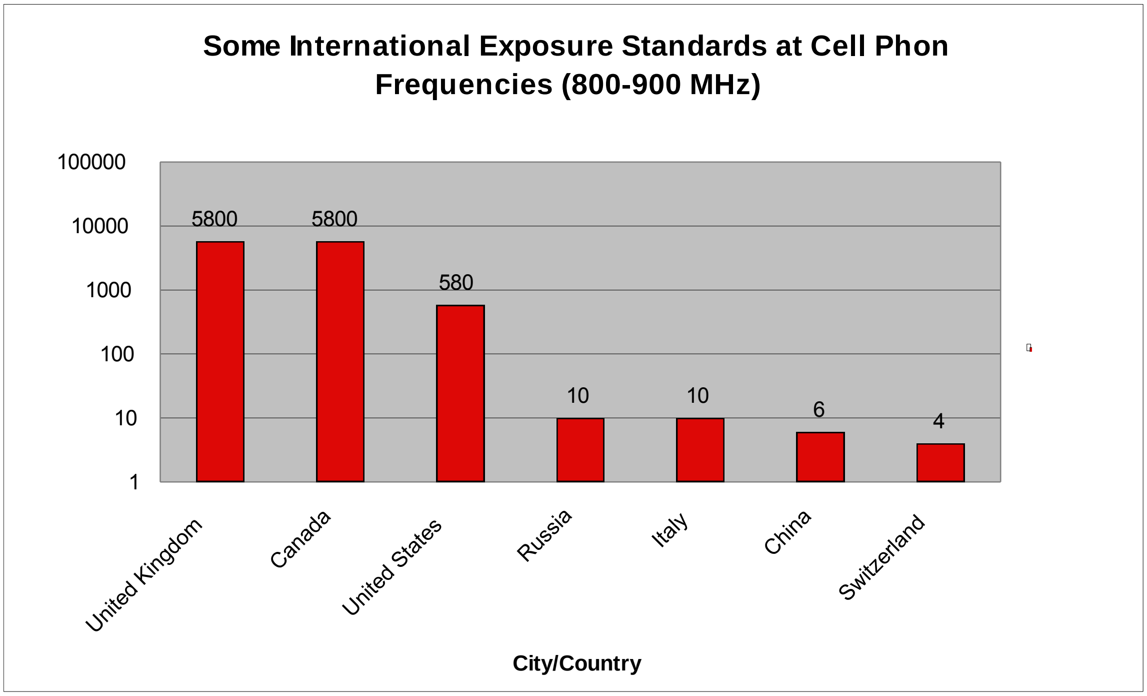
ICNRP maintains that guidelines for limiting exposure have been developed following a thorough review of all published scientific literature (ICNIRP, 1998).

"The criteria applied in the course of the review were designed to evaluate the credibility of the various reported findings (Repacholi and Stolwijk 1991; Repacholi and Cardis 1997); only established effects were used as the basis for the proposed exposure restrictions. Induction of cancer from long-term EMF exposure was not considered to be established, and so these guidelines are based on short-term, immediate health effects such as stimulation of peripheral nerves and muscles, shocks and burns caused by touching conducting objects, and elevated tissue temperatures resulting from absorption of energy during exposure to EMF. In the case of potential long-term effects of exposure, such as an increased risk of cancer, ICNIRP concluded that available data are insufficient to provide a basis for setting exposure restrictions, although epidemiological research has provided suggestive, but unconvincing, evidence of an association between possible carcinogenic effects and exposure at levels of 50/60 Hz magnetic flux densities substantially lower than those recommended in these guidelines. In-vitro effects of short-term exposure to ELF or ELF amplitude-modulated EMF are summarized. Transient cellular and tissue responses to EMF exposure have been observed, but with no clear exposure–response relationship. These studies are of limited value in the assessment of health effects because many of the responses have not been demonstrated in vivo. Thus, in-vitro studies alone were not deemed to provide data that could serve as a primary basis for assessing possible health effects of EMF. " (ICNIRP, 1998) http://www.icnirp.de

Guidelines and Limits (Other Countries)

On the other hand, some countries in the world have established new, low-intensity based exposure standards that respond to studies reporting effects that do not rely on heating. Consequently, new exposure guidelines are hundreds or thousands of times lower than those of IEEE and ICNIRP. Table 3.3 shows some of the countries that have lowered their limits, for example, in the cell phone frequency range of 800 MHz to 900 MHz. The levels range from 10 microwatts per centimeter squared in Italy and Russia to 4.2 microwatts per centimeter squared in Switzerland. In comparison, the United States and Canada limit such exposures to only 580 microwatts per centimeter squared (at 870 MHz) and then averaged over a time period (meaning that higher exposures are allowed for shorter times, but over a 30 minute period, the average must be 580 microwatts per centimeter squared or less at this frequency). The United Kingdom allows one hundred times this level, or 5800 microwatts per centimeter squared. Higher frequencies have higher safety limits, so that at 1000 MHz, for example, the limit is 1000 microwatts per centimeter squared (in the United States). Each individual frequency in the radiofrequency radiation range needs to be calculated. These are presented as reference points only. Emerging scientific evidence has encouraged some countries to respond by adopting planning targets, or interim action levels that are responsive to low-intensity or non-thermal radiofrequency radiation bioeffects and health impacts.

Table 3.3 Some International Exposure Standards at Cell Phone Frequencies



Professional bodies from technical societies like IEEE and ICNIRP continue to support "thermal-only" guidelines routinely defend doing so a) by omitting or ignoring study results reporting bioeffects and adverse impacts to health and wellbeing from a very large body of peer-reviewed, published science because it is not yet "proof" according to their definitions; b) by defining the proof of "adverse effects" at an impossibly high a bar (scientific proof or causal evidence) so as to freeze action; c) by requiring a conclusive demonstration of both "adverse effect" and risk before admitting low-intensity effects should be taken into account; e) by ignoring low-intensity studies that report bioeffects and health impacts due to modulation; f) by conducting scientific reviews with panels heavily burdened with industry experts and under-represented by public health experts and independent scientists with relevant low-intensity research experience; g) by limiting public participation in standard-setting deliberations; and other techniques that maintain the status quo.

Much of the criticism of the existing standard-setting bodies comes because their contributions are perceived as industry-friendly (more aligned with technology investment and dissemination of new technologies) rather than public health oriented. The view of the Chair of the latest IEEE standard-setting ICES Eleanor Adair is made clear by Osepchuk and Petersen (2003) who write in the abstract of their paper "*her goal and the goal of ICES is to establish rational standards that will make future beneficial applications of RF energy credible to humanity.*" Authors Osepchuk and Petersen note that *"(I)t is important that safety standards be rational and avoid excessive safety margins."* The authors specifically dismiss the body of evidence for low-intensity effects with *"(A)lthough the literature reporting "athermal" bioeffects of exposure to* *microwave/RF energy (other than electrostimulation) is included in the review process, it has been found to be inconsistent and not useful for purposes of standard-setting."*

This report addresses the substantial body of evidence reporting low-intensity effects from electromagnetic fields (both power-frequency fields in the ELF range, and radiofrequency/microwave fields at exposure levels that do not involve any heating. It also addresses the inconsistency in the literature quoted as the basis for retaining thermal-only exposure standards (see particularly the Genotoxics Section 6 where half of more of the published papers report negative effects and half positive effects).

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Osepchuk JM Petersen RC. 2003. Historical Review of RF Exposure Standards and the International Committee on Electromagnetic Safety (ICES). Bioelectromagnetics Supplement 6:S7-16. Osepchuk is a former employee of Raytheon. Petersen is a former employee of Bell Labs and Lucent Technologies. Both are independent industry consultants in their retirement.

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 4

Evidence for Inadequacy of the Standards

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September 2012

I. Introduction

Evidence for judging the adequacy (or inadequacy) of the existing ICNIRP and IEEE C95.1 radiofrequency radiation standards can be taken from many relevant sources. The ICNIRP standards are similar to the IEEE (except for the new C95.1 -2006) revisions by IEEE SC-4), and these discussions can be used to evaluate both sets of public exposure standards for adequacy (or inadequacy).

An important screen for assessment of how review bodies conduct their science reviews and resulting conclusions on the adequacy of ELF and RF exposure limits depends on embedded assumptions. The singularly most important embedded assumption is whether these bodies assume from the beginning that only conclusive scientific evidence (proof) will be sufficient to warrant change; or whether actions should be taken on the basis of a growing body of evidence which provides early but consequential warning of (but not yet proof) of possible risks.

As a result of current international research and scientific discussion on whether the prevailing RF and ELF standards are adequate for protection of public health, there are many recent developments prior to 2007 to provide valuable background on the uncertainty about whether current standards adequately protect the public. Since 2007, there are important new milestone publications that underscore the critical need to update public safety limits. These newer documents calling for review and updating are based on a deluge of new scientific studies reporting effects at non-thermal, low-intensity ELF and RF exposure levels. There is little doubt that bioeffects and adverse health effects are occurring at lower-than-safety limit levels, meaning the existing protections are inadequate.

II. United States Government Accountability Office

The US Government Accountability Office published a report in 2012 urging the US Federal Communications Commission to revisit the outdated safety standards for the exposures from wireless devices. (US GAO, 2012)

The rapid adoption of mobile phones has occurred amidst controversy over whether the technology poses a risk to human health as a result of long-term exposure to RF energy from mobile phone use. FCC and FDA share regulatory responsibilities for mobile phones. GAO was asked to examine several issues related to mobile phone health effects and regulation. Specifically, this report addresses:

> (1) what is known about the health effects of RF energy from mobile phones and what are current research activities,
> (2) how FCC set the RF energy exposure limit for mobile phones, and
> (3) federal agency and industry actions to inform the public about health issues related to mobile phones, among other things.

GAO reviewed scientific research; interviewed experts in fields such as public health and engineering, officials from federal agencies, and representatives of academic institutions, consumer groups, and the mobile phone industry; reviewed mobile phone testing and certification regulations and guidance; and reviewed relevant federal agency websites and mobile phone user manuals.

The Report noted that the FCC's RF energy exposure limit may not reflect the latest research. Redundant and overlapping jurisdiction over the setting of public safety limits is highlighted where the GAO Report notes:

> *"FCC told GAO that it relies on the guidance of federal health and safety agencies when determining the RF energy exposure limit, and to date, none of these agencies have advised FCC to change the limit. However, FCC has not formally asked these agencies for a reassessment. By not formally reassessing it's current limit, FCC cannot ensure it is using a limit that reflects the latest research on RF energy exposure. FCC has also not reassessed it's testing requirements to ensure that they identify the maximum RF energy exposure a user could experience. Some consumers may use mobile phones against the body, which FCC does not currently test, and could result in RF energy exposure higher than the FCC limit." (US GAO, 2012)*

The GAO Report recommends to the FCC that it formally reassess, and, if appropriate, change it's current RF energy exposure limit and mobile phone testing requirements related to likely usage configurations, particularly when phones are held against the body.

FCC noted that a draft document that is now under consideration by the FCC has the potential to address GAO's recommendations. (US GAO, 2012)

III. International Agency for Research on Cancer - World Health Organization Classifies Radiofrequency Radiation as 2B Possible Human Carcinogen

In 2011, a group of 30 researchers, scientists and medical doctors were invited to participate in an assessment of the scientific literature on radiofrequency radiation carcinogenicity in Lyon, France. Under the auspices of IARC, they conducted a comprehensive scientific assessment of RF studies and determined:

> *"In view of the limited evidence in humans and in experimental animals, the Working Group classified RF- EMF as "possibly carcinogenic to humans" (Group 2B). This evaluation was supported by a large majority of Working Group members." (Baan et al, 2011)*
>
> *"(T)he Working Group concluded that the (Interphone Final Report) findings could not be dismissed as reflecting bias alone, and that a causal interpretation between mobile phone RF-EMF exposure and glioma is possible. A similar conclusion was drawn from these two studies for acoustic neuroma, although the case numbers were substantially smaller than for glioma." (Baan et al, 2011)*

It is important to recognize that the IARC RF Working Group did not find the evidence insufficient to classify (Group 3) or not a carcinogen (Group 4). Both of these possible outcomes to the scientific assessment could have rendered a substantially weaker conclusion. Where there has been the necessity of a virtual scientific paradigm shift to accommodate ANY consideration of both ELF-EMF and RFR to the status where legitimate scientific attention is achieved is a notable achievement. There is a very high bar set to show that non-chemical carcinogens warrant IARC carcinogenicity evaluation - it greatly exceeds that necessary for chemicals and other toxins.

IV. World Health Organization *INTERPHONE* Study on Mobile Phone Cancer Risk

In 2010, the World Health Organization released the final results of it's investigation on cell phones and cancer. (INTERPHONE Study Group, 2010) The ten-year long World Health Organization *INTERPHONE Study* confirms previous reports showing what many experts have warned – that regular use of a cell phone by adults can significantly increase the risk of glioma by 40% with 1640 hours or more of use (this is about one-half hour per day over ten years). Tumors were more likely to occur on the side of the head most used for calling. The risk increases to 96% for adults with ipsilateral cell phone use (when the cell phone is used predominantly on one side of the head). The study appears in the International Journal of Epidemiology. Thirteen teams from countries around the world combined their results. Only the glioma findings were released (final results on acoustic neuroma and parotid tumors are not yet published.

A comprehensive and technically reliable description of the *INTERPHONE* study findings is provided within the International Agency for Research on Cancer, 2011 RF Monograph as part of the publication in Lancet Oncology on IARC's classification of radiofrequency radiation as a 2B Possible Human Carcinogen. Results of the *INTERPHONE* Study were highly scrutinized by IARC, and influenced the classification of RF based on the cell phone-brain cancer findings of *INTERPHONE*.

From Baan et al, 2011:

> *"The INTERPHONE study, a multi-centre case-control study, is the largest investigation so far of mobile phone use and brain tumours, including glioma, acoustic neuroma, and meningioma.The pooled analysis included 2708 glioma cases and 2972controls(participation rates64% and 53%, respectively). Comparing those who ever used mobile phones with those who never did yielded an odds ratio (OR) of 0·81 (95% CI 0·70–0·94). In terms of cumulative call time, ORs were uniformly below or close to unity for all deciles of exposure except the highest decile (>1640 h of use), for which the OR for glioma was 1·40 (95% CI 1·03–1·89). There was suggestion of an increased risk for ipsilateral exposure(on the same side of the head as the tumour) and for tumours in the temporal lobe, where RF exposure is highest. Associations between glioma and cumulative specific energy absorbed at the tumour location were examined in a subset of 553 cases that had estimated RF doses.10 The OR for glioma increased with increasing RF dose for exposures 7 years or more before diagnosis, whereas there was no association with estimated dose for exposures less than 7 years before diagnosis.*
>
> *A Swedish research group did a pooled analysis of two very similar studies of associations between mobile and cordless phone use and glioma, acoustic neuroma, and meningioma.9 The analysis included 1148 glioma cases (ascertained 1997–2003) and 2438 controls,obtained through cancer and population registries,*

> *respectively. Self-administered mailed questionnaires were followed by telephone interviews to obtain information on the exposures and covariates of interest, including use of mobile and cordless phones (response rates 85% and 84%, respectively). Participants who had used a mobile phone for more than 1 year had an OR for glioma of 1.3 (95% CI 1·1–1·6). The OR increased with increasing time since first use and with total call time, reaching 3.2 (2·0–5·1) for more than 2000 h of use. Ipsilateral use of the mobile phone was associated with higher risk. Similar findings were reported for use of cordless phones.*
>
> *Although both the INTERPHONE study and the Swedish pooled analysis are susceptible to bias—due to recall error and selection for participation— the Working Group concluded that the findings could not be dismissed as reflecting bias alone, and that a causal interpretation between mobile phone RF-EMF exposure and glioma is possible. A similar conclusion was drawn from these two studies for acoustic neuroma, although the case numbers were substantially smaller than for glioma. Additionally, a study from Japan (11) found some evidence of an increased risk for acoustic neuroma associated with ipsilateral mobile phone use.*
>
> *(Baan et al, 2011)*

No that no increased risk was detected overall. But this is not unexpected. No exposures to carcinogens that cause solid tumors like brain cancer or lung cancers, for example from tobacco and asbestos have ever been shown to significantly increase cancer risk in people with such short duration of exposure. The latency period for brain cancer is 15-30 years.

The final INTERPHONE results support findings of several research groups who have published studies reporting that continuing use of a mobile phone increases risk of brain cancer. We would not expect to see substantially increased brain tumor risk for most cancer-causing agents except in the longer term (10 year and longer) as is the case here in the population of regular cell phone users. Further, the participants included in this study were 30-59 years old, excluding younger and older users. Use of cordless phones was neglected in the analysis. Radiofrequency radiation from some cordless phones can be as high as mobile phones in some countries, so excluding such use would underestimate the risk for brain tumors and other cancers.

For public health experts and members of the public who looked to IARC for further clarification of the scope of this 2B Possible Human Carcinogen designation, Dr. Baan replied to informal queries that:

> *"Although the key information came from mobile telephone use, the Working Group considered that the three types of exposure entail basically the same type of radiation, and decided to make an overall evaluation on RF-EMF, covering the whole radiofrequency region of the electromagnetic spectrum.*
>
> *In support of this, information from studies with experimental animals showed that effects on cancer incidence and cancer latency were seen with exposures to different frequencies within the RF region.*
>
> *So the classification 2B, possibly carcinogenic, holds for all types of radiation within the radiofrequency part of the electromagnetic spectrum, including the radiation emitted by base-station antennas, radio/ TV towers, radar, Wi-Fi, smart meters, etc." (Personal communication of Dr. Robert Baan to Connie Hudson, August 29, 2011)*

V. President's Cancer Panel Report of 2010

The United States President's Cancer Panel Report (2010) includes important and unprecedented recognition of non-ionizing radiation as a possible carcinogen deserving of further research and possible public health action. The Report found "the true burden of environmentally induced cancers has been grossly underestimated" and strongly urged action to reduce peoples' widespread exposures to carcinogens. The 240-page report issued for 2008-2009 by a panel of experts that report to the US president indicate that environmental factors are underestimated in cancer prevention. The Report specifically addresses the link between cell phones and cancer. The Panel recommends that people reduce their cell phone exposure, even when absolute proof of harm is not yet available.

Research Recommended by Presidents Cancer Panel

- Resolve controversies regarding the safety or harm of low doses of various forms of radiation in adults and children. Identify circumstances under which low- dose radiation may have a hormetic effect.

- Develop radiation dose and risk estimates that better reflect the current and future U.S. population. Existing dose and risk estimates have been based on adult males; estimates should account for population diversity, including children. In addition, develop medical radiation risk estimates that are not based on acute doses received by atomic bomb survivors.

- Expand research on possible harmful effects of cell phone use, especially in children. Cell phone use still is relatively recent, and studies to date have had mixed findings; most involve users of older equipment. Findings from cohort studies now underway are anticipated, but longer-term studies of individuals using current equipment are needed.

- Conduct additional research on possible links between electromagnetic fields (EMF) and cancer; identify mechanism(s) of EMF carcinogenesis.

- Monitor changing patterns of radiation exposure.

- Raise the priority of and investment in research to develop non-toxic products anD processes.

- Develop, test, and evaluate prevention communication strategies and interventions, especially in high-risk occupations and populations.

(National Cancer Institute, 2010)

VI. World Health Organization Research Agenda for Radiofrequency Fields (2010)

In 2010, the WHO produced a research agenda to address growing scientific questions and public concern about health effects of radiofrequency radiation, particularly with the explosive rise in exposures from new telecommunications technologies. It replaced a 2006 research agenda developed by the International EMF Project.

> *"Telecommunication technologies based on radiofrequency (RF) transmission, such as radio and television, have been in widespread use for many decades. However, there are numerous new applications for the broadcast and reception of RF waves and the use of RF devices such as mobile phones is now ubiquitous.*
>
> ***The attendant increased public exposure to RF fields has made its effects on human health a topic of concern for scientists and the general public.***
> *(emphasis added)*
>
> *To respond to these concerns, an important research effort has been mounted over the past decade and many specific questions about potential health effects of RF fields have already been investigated by scientists around the world. Nonetheless, several areas still warrant further investigation and the rapid evolution of technology in this field is raising new questions."* *(WHO, 2010)*
>
> *"This Research Agenda is developed ahead of the major hazard/health risk evalu-*

> *ations that the IARC and WHO are due to carry out over the next two years. It focuses on identifying short- and long-term research needs that will enable more complete health risk assessments to be undertaken and communicated more effectively to the public."* *(WHO, 2010)*

Recommendations of the WHO Research Agenda for Radiofrequency Fields are as follows. This section is necessarily extensive to document the advice of experts at WHO by 2010 in recognizing radiofrequency radiation has the potential to result in global health impacts; even if very slow to implement precautionary advice to the European Commission and member countries.

Priority: Epidemiology

High - Prospective cohort studies of children and adolescents with outcomes including behavioural and neurological disorders and cancer

Rationale: As yet, little research has been conducted in children and adolescents and it is still an open question whether children are more susceptible to Rf EMF since the brain continues to develop during childhood and adolescence. also, children are starting to use mobile phones at a younger age. given the existence of large-scale cohort studies of mothers and children with follow-up started during or before pregnancy, an Rf sources component could be added at a reasonably low cost. Billing records for mobile phones are not valid for children, therefore the prospective collection of exposure data is needed. for neuropsychological studies, one challenge is to distinguish the "training" of motor and neu- ropsychological skills caused by the use of a mobile phone from the effects of the Rf field. any future study should try to address this issue. in any case it should be of longitudinal design, thereby allowing the study of several outcomes and changes in technology and the use of mobile phones as well as other sources of Rf eMf exposure, such as wireless laptops.

High - Monitoring of brain tumour incidence trends through well-established population-based cancer registries, if possible combined with population exposure data

Rationale: If there is a substantial risk associated with mobile phone use, it should be observable in data sources of good quality. such time trend analyses can be performed quite quickly and inexpensively. By using modern statistical techniques for analysing popu- lation data it should be possible to link changes in exposure prevalence in the population to the incidence of brain tumours and, if high-quality surveillance data are available, the incidence of other diseases at the population level. given the shortcomings in the exposure assessment and participation of previous studies based on individual data, an ecological study would have benefits that may outweigh its limitations.

Other - case-control studies of neurological diseases provided that objective exposure data and confounder data are available and reasonable participation is achieved

Rationale: Neurological endpoints, such as alzheimer disease and Parkinson disease, may be as biologically plausible as brain cancer and an increased risk would have a major public health impact. This study could give an early warning sign that can be elaborated further in the prospective cohort studies. an analysis of time-trends in neurological disease could also serve as an early warning sign. However, a feasibility study would be necessary in order to determine whether a good quality case-control study could be carried out.

Priority: Human studies

High - further RF EMf provocation studies on children of different ages

Rationale: current research has focused primarily on adolescents; very little is known about possible effects in younger children. longitudinal testing at different ages, for example by studying children already participating in current cohort studies, is recommended. This would allow consideration of the influence of potentially confounding factors such as lifestyle.

High - Provocation studies to identify neurobiological mechanisms underlying possible effects of RF on brain function, including sleep and resting EEG

Rationale: These studies should include validation of these effects using a range of brain imaging methods. They should also include studies investigating possible thresholds and dose-response relationships at higher exposure levels such as those encountered during occupational exposure.

Priority: Animal studies

High - Effects of early-life and prenatal RF exposure on development and behaviour

Rationale: There is still a paucity of information concerning the effects of prenatal and early life exposure to RF EMf on subsequent development and behaviour. Such studies are regarded as important because of the widespread use of mobile phones by children and the increasing exposure to other RF sources such as wireless local area networks (Wlans) and the reported effects of RF EMf on the adult EEG. further study is required which should include partial (head only) exposure to mobile phones at relatively high specific absorption rate (SAR) levels.

High - effects of RF exposure on ageing and neurodegenerative diseases

Rationale: age-related diseases, especially neurodegenerative diseases of the brain such as alzheimer disease and Parkinson disease, are increasingly prevalent and are therefore an important public health issue. Mobile phone use typically involves repeated Rf eMf exposure of the brain; a recent study has suggested that this type of exposure could affect alzheimer disease in a transgenic mouse model for this condition (arendash et al., 2010). There are a few ongoing studies of possible Rf eMf effects on neurodegenerative diseases but further studies are required to investigate this subject more fully.

Other research needs - Effects of RF exposure on reproductive organs

Rationale: The available data concerning possible effects of Rf eMf from mobile phones on male fertility are inconsistent and their quality and exposure assessments are weak. in vivo studies on fertility should consider effects on both males and females and investigate a range of relevant endpoints including Rf eMf effects on the development and function of the endocrine system.

Priority: Cellular studies

Other - Identify optimal sets of experimental tests to detect cellular response after exposure to new RF technologies and co-exposures of RF EMF with environmental agents

Rationale: a number of in vitro studies investigating the effects of exposure to mobile phone frequencies/signals, or co-exposures of RF EMf with chemical or physical agents, have been published in the last fifteen years. Results obtained have been inconsistent and contradictory, not least because of the use of a large variety of cell types and study approaches. a set of highly sensitive, well-harmonized cellular and molecular methods should be developed in order to screen the toxic potential of new types of RF signals used in new technologies and of co-exposures of RF EMf and environmental agents – especially those suspected to have toxic effects. This research must be multicentred in order to allow the widest possible acceptance and application of this screening tool.

Other - further studies on the influence of genetic background and cell type: possible effects of mobile phone type Rf exposure on a variety of cell types using newer, more sensitive methods less susceptible to artefact and/or bias

Rationale: More rigorous quantitative methods should be employed in the evaluation of positive results that suggest a specific cell type response, e.g. of embryonic cells (Czyz et al., 2004; Franzellitti et al., 2010), raising the possibility that RF impacts specific cell subpopulations or cell types. These studies should include a variety of cell types such as stem cells and cells with altered genetic backgrounds.

Priority: Mechanisms: none

Priority: Dosimetry

High - Assess characteristic RF EMF emissions, exposure scenarios and corresponding exposure levels for new and emerging RF technologies; also for changes in the use of established technologies

Rationale: The work should address the latest developments in areas such as mobile/cordless phones, wireless data networking, asset tracking and identification, wireless transfer of electrical power and body imaging/scanners. it should also consider the possible combined effect of exposure to multiple sources. This will allow exposures from new devices/scenarios to be compared with those that are more familiar and with exposure guidelines for risk communication purposes. This information will also be of value for exposure assessment in epidemiological studies and in the design of biological exposure systems.

High - quantify personal exposures from a range of RF sources and identify the determinants of exposure in the general population

Rationale: The quantification of personal exposure from a range of RF sources will provide valuable information for risk assessment and communication, and for the development of future epidemiological research. it is particularly useful for global exposure assessment in view of the upcoming WHO health risk assessment. The study will also provide baseline data for identification of any changes in the level of exposure and the dominant contributing factors over time. subgroup analyses should be carried out to identify any influence from demographic aspects of the user as well as the microenvironment in which the exposure occurs. exposure metrics should also be considered, especially in combining localized exposures from body-worn devices and whole-body exposures.

Other research needs - Monitoring of personal exposure of Rf workers

Rationale: The exposure patterns of both workers and the general public change continuously, mainly due to the development of new RF technologies. However, workers encounter industrial sources and exposure situations that lead to much higher energy deposition in the body. When epidemiological studies on RF workers are performed, it is imperative to monitor adequately their RF exposure. new instruments are needed to address the lack of adequate measurement tools for evaluating this type of exposure e.g. portable devices suitable for measuring different frequencies and waveforms. in addition, a study of the feasibility of monitoring the personal exposure of RF workers is required for future epidemiological studies. such studies would be facilitated by the production of a job exposure matrix (JeM) for RF workers – in which job designations can be characterized by their exposure. (WHO, 2010)

VII. National Academy of Sciences, National Research Council (2008)

The U.S. Food and Drug Administration (FDA) of the Department of Health and Human Services asked the National Academies to organize a workshop of national and international experts to identify research needs and gaps in knowledge of biological effects and adverse health outcomes of exposure to radiofrequency (RF) energy from wireless communications devices. To accomplish this task, the National Academies appointed a seven member committee to plan the workshop.1

Following the workshop, the committee was asked to issue a report based on the presentations and discussions at the workshop that identified research needs and current gaps in knowledge. The committee's task did not include the evaluation of health effects or the generation of recommendations relating to how the identified research needs should be met.

For the purposes of this report, the committee defines research needs as research that will increase our understanding of the potential adverse effects of RF energy on humans. Research gaps are defined as areas of research where the committee judges that scientific data that have potential value are presently lacking, but that closing of these gaps is either ongoing and results should be awaited before judgments are made on further research needs, or the gaps are not judged by the committee to be of as high a priority with respect to directly addressing health concerns at this time.

1. Committee on Identification of Research Needs Relating to Potential Biological or Adverse Health Effects of Wireless Communications Devices.

These needs and gaps are committee judgments derived from the workshop presentations and discussions, and the report does not necessarily reflect the views of the FDA, individual workshop speakers, or other workshop participants.

The committee judged that important research needs included, in order of appearance in the text, the following:

> - Characterization of exposure to juveniles, children, pregnant women, and fetuses from personal wireless devices and RF fields from base station antennas.
>
> - Characterization of radiated electromagnetic fields for typical multiple- element base station antennas and exposures to affected individuals.
>
> - Characterization of the dosimetry of evolving antenna configurations for cell phones and text messaging devices.
>
> - Prospective epidemiologic cohort studies of children and pregnant women.
>
> - Epidemiologic case-control studies and childhood cancers, including brain cancer.
>
> - Prospective epidemiologic cohort studies of adults in a general population and retrospective cohorts with medium to high occupational exposures.
>
> - Human laboratory studies that focus on possible adverse effects on electroencephalography2 activity and that include a sufficient number of subjects.
>
> - Investigation of the effect of RF electromagnetic fields on neural networks.
>
> - Evaluation of doses occurring on the microscopic level.
>
> - Additional experimental research focused on the identification of potential biophysical and biochemical/molecular mechanisms of RF action.
>
> (NAS-NRC, 2008)

VIII. World Health Organization Draft Framework for Electromagnetic Fields

The International EMF Project was established by WHO in 1996. Its mission was to *"pool resources and knowledge concerning the effects of exposure to EMF and make a concerted effort to identify gaps in knowledge, recommend focused research programmes that allow better health risk assessments to be made, conduct updated critical reviews of the scientific literature, and work towards an international consensus and solutions on the health concerns."* (WHO September 1996 Press Release - Welcome to the International EMF Project)

The stated role of the WHO Precautionary Framework on EMF Health Risk Research (Radiation and Environment Health) has termed its objectives as follows;

- to anticipate and respond to possible threats before introduction of an agent or technology
- to address public concerns that an uncertain health risk is minimized after introduction of an agent
- to develop and select options proportional to the degree of scientific certainty, the severity of harm, the size and nature of the affected population and the cost.

The role of WHO is advisory only to the countries of Europe but it is an important function and can significantly affect decision-making on public health issues. It provides analysis and recommendations on various topics of health and environment, for consideration by member countries of the EU. Given the EU Article 174 policy requires a precautionary approach to judging health and environmental risks, and given that the charter of WHO is to serve the needs of the EU, one would think it essential that the WHO EMF Program health criteria results should be guided by and tailored to compliance with Article 174. This needs to occur in the assessment of the scientific literature (e.g., not requiring studies to provide scientific proof or causal scientific evidence but paying attention to and acting on the evidence, and the trend of the evidence at hand) and in its environmental health criteria recommendations. If the WHO EMF Program instead chooses to use the definitions of adverse impact and risk based on reacting to nothing short of conclusive scientific evidence, it fails to comply with the over-arching EU principle of health.

The World Health Organization has issued a draft framework to address the adequacy of scientific information, and accepted definitions of bioeffects, adverse health effect and hazard (WHO EMF Program Framework for Developing EMF Standards, Draft, October 2003). These definitions are not subject to the whim of organizations preparing public exposure standard recommendations. The WHO definition states that:

> "(A)nnoyance or discomforts caused by EMF exposure may not be pathological per se, but, if substantiated, can affect the physical and mental well-being of a

> person and the resultant effect may be considered as an adverse health effect. A health effect is thus defined as a biological effect that is detrimental to health or well-being. According to the WHO Constitution, health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity." **www.who.int/peh-emf**

IX. The European Union Treaties Article 174

The EU policy (Article 174-2) requires that the precautionary principle be the basis for environmental protection for the public, and that protecting public health and taking preventative action before certainty of harm is proven is the foundation of the Precautionary Principle. It is directly counter to the principles used by ICNIRP and IEEE in developing their recommendations for exposure standards. Both bodies require proof of adverse effect and risk before amending the exposure standards; this Treaty requires action to protect the public when a reasonable suspicion of risk exists (precautionary action).

Article 174 (2) [ex Article 130r]

1. Community policy on the environment shall contribute to pursuit of the following objectives:
—preserving, protecting and improving the quality of the environment;
—protecting human health;
—prudent and rational utilisation of natural resources;
—promoting measures at international level to deal with regional or worldwide environmental problems.

2. Community policy on the environment shall aim at a high level of protection taking into account the diversity of situations in the various regions of the Community. It shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should as a priority be rectified at source and that the polluter should pay. In this context, harmonization measures answering environmental protection requirements shall include, where appropriate, as a safeguard clause allowing Member States to take provisional measures, for non-economic environmental reasons, subject to a Community inspection procedure.

3. In preparing its policy on the environment, the Community shall take account of:

—available scientific and technical data;
—environmental conditions in the various regions of the Community;

—the potential benefits and costs of action or lack of action;
—the economic and social development of the Community as a whole and the balanced development of its regions.

http://www.law.harvard.edu/library/services/research/guides/international/eu/eu_legal_research_treaties.php

X. WHO ELF Environmental Health Criteria Monograph, June 2007

In 2007. the WHO EMF Program released its ELF Health Criteria Monograph and held a workshop in Geneva, Switzerland June 20-21st.

ELF Health Criteria Monograph

12.6 Conclusions

Acute biological effects have been established for exposure to ELF electric and magnetic fields in the frequency range up to 100 kHz that may have adverse consequences on health. Therefore, exposure limits are needed. International guidelines exist that have addressed this issue. Compliance with these guidelines provides adequate protection.

Consistent epidemiological evidence suggests that chronic low-intensity ELF magnetic field exposure is associated with an increased risk of childhood leukaemia. ***However, the evidence for a causal relationship is limited, therefore exposure limits based upon epidemiological evidence are not recommended, but some precautionary measures are warranted.*** (emphasis added).

The Monograph finds no reason to change the designation of EMF as a 2B (Possible) Human Carcinogen as defined by the International Agency for Cancer Research (IARC). In finding that ELF-EMF is classifiable as a possible carcinogen, it is inconsistent to conclude that no change in the exposure limits is warranted. If the Monograph confirms, as other review bodies have, that childhood leukemia occurs at least as low as the 3 mG to 4 mG exposure range, then ICNIRP limits of 1000 mG for 50 Hz and 60 Hz ELF exposures are clearly too high and pose a risk to the health of children.

The WHO Fact Sheet summarizes some of the Monograph findings but adds further recommendations.

"Potential long-term effects"

Much of the scientific research examining long-term risks from ELF magnetic field exposure has focused on childhood leukaemia. In 2002, IARC published a monograph classifying ELF magnetic fields as "possibly carcinogenic to humans. This classification was based on pooled analyses of epidemiological studies demonstrating a consistent *pattern of a two-fold increase in childhood leukaemia associated with average exposure to residential power-frequency magnetic field above 0.3 to 0.4 µT.* ***The Task Group concluded that additional studies since then do not alter the status of this classification."*** *(emphasis added)*

"International exposure guidelines*"*

"Health effects related to short-term, high-level exposure have been established and form the basis of two international exposure limit guidelines (ICNIRP, 1998; IEEE, 2002). At present, these bodies consider the scientific evidence related to possible health effects from long-term, low-level exposure to ELF fields insufficient to justify lowering these quantitative exposure limits."

"Regarding long-term effects, given the weakness of the evidence for a link between exposure to ELF magnetic fields and childhood leukaemia, the benefits of exposure reduction on health are unclear. In view of this situation, the following recommendations are given:

> *1) Government and industry should monitor science and promote research programmes to further reduce the uncertainty of the scientific evidence on the health effects of ELF field exposure. Through the ELF risk assessment process, gaps in knowledge have been identified and these form the basis of a new research agenda.*
>
> *2) Member States are encouraged to establish effective and open communication programmes with all stakeholders to enable informed decision-making. These may include improving coordination and consultation among industry, local government, and citizens in the planning process for ELF EMF-emitting facilities.*
>
> *3) When constructing new facilities and designing new equipment, including appliances, low-cost ways of reducing exposures may be explored. Appropriate exposure reduction measures will vary from one country to another. However, policies based on the adoption of arbitrary low exposure limits are not warranted."*

The last bullet in the WHO ELF Fact Sheet does not come from the Monograph, nor is it consistent with conclusions of the Monograph. The Monograph does call for prudent avoidance measures, one of which could reasonably be to establish numeric planning targets or interim limits for new and upgraded transmission lines and appliances used by children, for example. Countries should not be dissuaded by WHO staff, who unlike the authors of the Monograph, go too far in defining appropriate boundaries for countries that may wish to implement prudent avoidance in ways that best suit their population needs, expectations and resources. www.who.int/peh-emf/project/en

XI. World Health Organization Report on Children's Health and Environment

Environmental Issue Report Number 29 from the World Health Organization (2002) cautions about the effects of radiofrequency radiation on children's health. As part of a publication on "Children's Health and Environment: A Review of Evidence" the World Health Organization (WHO) wrote:

> *"The possible adverse health effects in children associated with radiofrequency fields have not been fully investigated."*
>
> *"Because there are suggestions that RF exposure may be more hazardous for the fetus and child due to their greater susceptibility, prudent avoidance is one approach to keeping children's exposure as low as possible."*
>
> *"Further research is needed to clarify the potential risks of ELF-EMF and radiofrequency fields for children's health."*

XII. International Agency for Research on Cancer (IARC)

A 2001 report by the WHO International Agency for Research on Cancer (IARC) concluded that ELF-EMF power frequency fields are a Category 2B (Possible) Human Carcinogen. These are power-frequency electromagnetic fields (50-Hz and 60-Hz electric power frequency fields).

The World Health Organization (WHO) is conducting the International Electromagnetic Fields (EMF) Project to assess health and environmental effects of exposure to static and time varying electric and magnetic fields in the frequency range of 1 – 300 gigahertz (GHz). Project goals include the development of international guidelines on exposure limits. This work will address radio and television broadcast towers, wireless communications transmission and telecommunications facilities, and associated devices such as mobile phones, medical and industrial equipment, and radars. It is a multi-year program that began in 1996 and will end in 2005. www.who.int/peh-emf

XIII. SCENIHR Opinion (European Commission Study of EMF and Human Health)

An independent Scientific Committee on newly emerging risks commissioned by the European Union released an update of its 2001 opinion on electromagnetic fields and human health in 2007. "The Committed addressed questions related to potential risks associated with interaction of risk factors, synergistic effects, cumulative effects, anti-microbial resistance, new technologies such as nanotechnologies, medical devices, tissue engineeringm blood products, fertility reduction, cancer of endocrine organs, physical hazards such as noise and electromagnetic fields and methodologies for assessing new risks." SCENIHR, 2007

SCENIHR Conclusions on Extremely low frequency fields (ELF fields)

> The previous conclusion that ELF magnetic fields are possibly carcinogenic, chiefly based on childhood leukaemia results, is still valid. There is no generally accepted mechanism to explain how ELF magnetic field exposure may cause leukaemia.
>
> For breast cancer and cardiovascular disease, recent research has indicated that an association is unlikely. For neurodegenerative diseases and brain tumours, the link to ELF fields remains uncertain. A relation between ELF fields and symptoms (sometimes referred to as electromagnetic hypersensitivity) has not been demonstrated.

SCENIHR Conclusions on Radiofrequency Radiation fields (RF fields)

> Since the adoption of the 2001 opinion, extensive research has been conducted regarding possible health effects of exposure to low intensity RF fields. This research has investigated a variety of possible effects and has included epidemiologic, in vivo, and in vitro research. The overall epidemiologic evidence suggests that mobile phone use of less than 10 years does not pose any increased risk of brain tumour or acoustic neuroma. For longer use, data are sparse, since only some recent studies have reasonably large numbers of long-term users. Any conclusion therefore is uncertain and tentative. From the available data, however, it does appear that there is no increased risk for brain tumours in long-term users, with the exception of acoustic neuroma for which there is limited evidence of a weak association. Results of the so-called Interphone study will provide more insight, but it cannot be ruled out that some questions will remain open.

SCENIHR Conclusions on Sensitivity of Children

> Concerns about the potential vulnerability of children to RF fields have been

> raised because of the potentially greater susceptibility of their developing nervous system; in addition, their brain tissue is more conductive than that of adults since it has a higher water content and ion concentration, RF penetration is greater relative to head size, and they have a greater absorption of RF energy in the tissues of the head at mobile telephone frequencies. Finally, they will have a longer lifetime exposure.
>
> Few relevant epidemiological or laboratory studies have addressed the possible effects of RF field exposure on children. Owing to widespread use of mobile phones among children and adolescents and relatively high exposures to the brain, investigation of the potential effect of RF fields in the development of childhood brain tumour is warranted. The characteristics of mobile phone use among children, their potential biological vulnerability and longer lifetime exposure make extrapolation from adult studies problematic.

There is an ongoing debate on possible differences in RF absorption between children and adults during mobile phone usage, e.g. due to differences in anatomy (Wiart et al. 2005, Christ and Kuster, 2005). Several scientific questions like possible differences of the dielectric tissue parameters remain open. The anatomical development of the nervous system is finished around 2 years of age, when children do not yet use mobile phones although baby phones have recently been introduced. Functional development, however, continues up to adult age and could be disturbed by RF fields.

XIV. Health Protection Agency (Formerly the NRPB - United Kingdom)

The National Radiation Protection Board or NRPB (2004) concluded, based on a review of the scientific evidence, that the most coherent and plausible basis from which guidance could be developed on exposures to ELF concerned weak electric field interactions in the brain and CNS (NRPB, 2004). A cautious approach was used to indicate thresholds for possible adverse health effects.

> *"Health Effects - It was concluded from the review of scientific evidence (NRPB, 2004b) that the most coherent and plausible basis from which guidance could be developed on exposures to ELF EMFs concerned weak electric field interactions in the brain and CNS (NRPB, 2004). A cautious approach was used to indicate thresholds for possible adverse health effects."*
>
> *"The brain and nervous system operate using highly complex patterns of \electrical signals. Therefore, the basic restrictions are designed to limit the*

> *electric fields and current densities in these tissues so as to not adversely affect their normal functioning. The adverse effects that might occur cannot easily be characterized according to presenting signs or symptoms of disease or injury. They represent potential changes to mental processes such as attention and memory, as well as to regulatory functions with in the body. Thus, the basic restrictions should not be regarded as precisely determined values below which no adverse health effects can occur and above which clearly discernible effects will happen. The do, however, indicate an increasing likelihood of effects occurring as exposure increases above the basic restriction values."*
>
> *"From the results of the epidemiological investigations, there remain concerns about a possible increased risk of child leukaemia associated with exposure to magnetic fields above about 0.4 uT (*4 mG*). In this regard, it is important to consider the possible need for further precautionary measures."*

This recent statement by the UK Health Protection Agency clearly indicates that the current guidelines may not be protective of public health. Yet, the reference levels used in the United Kingdom remain at 5000 mG for 50 Hz power frequency fields for occupational exposure and 1000 mG for public exposure.

XV. US Government Radiofrequency Interagency Working Group Guidelines Statement

The United States Radiofrequency Interagency Working Group (RFIAWG) cited concerns about current federal standards for public exposure to radiofrequency radiation in 1999 (Lotz, 1999 for the Radiofrequency Interagency Working Group)

> "*Studies continue to be published describing biological responses to nonthermal ELF-modulated RF radiation exposures that are not produced by CW (unmodulated) radiation. These studies have resulted in concern that 'exposure guidelines based on thermal effects, and using information and concepts (time-averaged dosimetry, uncertainty factors) that mask any differences between intensity-modulated RF radiation exposure and CW exposure, do not directly address public exposures, and therefore may not adequately protect the public."*

The United States government Federal Radiofrequency Interagency Working Group has reviewed the existing ANSI/IEEE RF thermal-based exposure standard upon which the FCC limit is based. This Working Group was made up of representatives from the US government's National Institute for Occupational Safety and Health (NIOSH), the

Federal Communications Commission (FCC), Occupational Health and Safety Administration (OSHA), the Environmental Protection Agency (US EPA), the National Telecommunication and Information Administration, and the US Food and Drug Administration (FDA).

On June 17, 1999, the RFIAWG issued a Guidelines Statement that concluded the present RF standard "may not adequately protect the public". The RFIAWG identified fourteen (14) issues that they believe are needed in the planned revisions of ANSI/IEEE RF exposure guidelines including **"**to provide a strong and credible rationale to support RF exposure guidelines". In particular, the RFIAWG criticized the existing standards as not taking into account chronic, as opposed to acute exposures, modulated or pulsed radiation (digital or pulsed RF is proposed at this site), time-averaged measurements that may erase the unique characteristics of an intensity-modulated RF radiation that may be responsible for reported biologic effects, and stated the need for a comprehensive review of long-term, low-level exposure studies, neurological-behavioral effects and micronucleus assay studies (showing genetic damage from low-level RF).

The existing federal standards may not be protective of public health in critical areas. The areas of improvement where changes are needed include: a) selection of an adverse effect level for chronic exposures not based on tissue heating and considering modulation effects; b) recognition of different safety criteria for acute and chronic exposures at non-thermal or low-intensity levels; c) recognition of deficiencies in using time-averaged measurements of RF that does not differentiate between intensity-modulated RF and continuous wave (CW) exposure, and *therefore may not adequately protect the public.*

As of 2007, requests to the RFIAWG on whether these issues have been satisfactorily resolved in the new 2006 IEEE recommendations for RF public safety limits have gone unanswered (BioInitiative Working Group, 2007).

XVI. United Kingdom - Parliament Independent Expert Group Report (Stewart Report)

The Parliament of the United Kingdom commissioned a scientific study group to evaluate the evidence for RF health and public safety concerns. In May of 2000, the United Kingdom Independent Expert Group on Mobile Phones issued a report underscoring concern that standards are not protective of public health related to both mobile phone use and exposure to wireless communication antennas.

Conclusions and recommendations from the Stewart Report (for Sir William Stewart) indicated that the Group has some reservation about continued wireless technology expansion without more consideration of planning, zoning and potential public health concerns. Further, the Report acknowledges significant public concern over community siting of mobile phone and other communication antennas in residential areas and near schools and hospitals.

> *"Children may be more vulnerable because of their developing nervous system, the greater absorption of energy in the tissue of the head and a longer lifetime of exposure."*
>
> *"The siting of base stations in residential areas can cause considerable concern and distress. These include schools, residential areas and hospitals."*
>
> *" There may be indirect health risks from living near base stations with a need for mobile phone operators to consult the public when installing base stations."*
>
> *"Monitoring should be expecially strict near schools, and that emissions of greatest intensity should not fall within school grounds."*
>
> *"The report recommends "a register of occupationally exposed workers be established and that cancer risks and mortality should be examined to determine whether there are any harmful effects."*
> (IEGMP, 2000)

XVII. Food and Drug Administration (US FDA)

The Food and Drug Administration announced on March 28, 2007 it is contracting with the National Academy of Science to conduct a symposium and issue a report on additional research needs related to possible health effects associated with exposure to radio frequency energy similar to those emitted by wireless communication devices. The National Academy of Sciences will organize an open meeting of national and international experts to discuss the research conducted to date, knowledge gaps, and additional research needed to fill those gaps. The workshop will consider the scientific literature and ongoing research from an international perspective in order to avoid duplication, and in recognition of the international nature of the scientific community and of the wireless industry.

Funding for the project will come from a Cooperative Research and Development Agreement (CRADA) between the Food and Drug Administration's Center for Devices and Radiological Health and the Cellular Telecommunications and Internet Association (CTIA). http://www.fda.gov/cellphones/index.html

XVIII. National Institutes for Health - National Toxicology Program

The National Toxicology Program (NTP) is a part of the National Institute for Environmental Health Sciences, National Institutes for Health. Public and agency comment has been solicited on whether to add radiofrequency radiation to its list of substances to be tested by NTP as carcinogens. In February 2000 the FDA made a recommendation to the NPT urging that RF be tested for carcinogenicity (www.fda.gov.us). The recommendation is based in part on written testimony stating:

> *" Animal experiments are crucial because meaningful data will not be available from epidemiological studies for many years due to the long latency period between exposure to a carcinogen and the diagnosis of a tumor.*
>
> *"There is currently insufficient scientific basis for concluding either that wireless communication technologies are safe or that they pose a risk to millions of users."*

> *"FCC radiofrequency radiation guidelines are based on protection from acute injury from thermal effects of RF exposure* and may not be protective *against any non-thermal effects of chronic exposures."*

In March of 2003, the National Toxicology Program issued a Fact Sheet regarding its toxicology and carcinogenicity testing of radiofrequency/microwave radiation. These studies will evaluate radiofrequency radiation in the cellular frequencies.

> "*The existing exposure guidelines are based on protection from acute injury from thermal effects of RF exposure. Current data are insufficient to draw definitive conclusions concerning the adequacy of these guidelines to be protective against any non-thermal effects of chronic exposures. "*

XIX. US Food and Drug Administration

In February of 2000, Russell D. Owen, Chief of the Radiation Biology Branch of the Center for Devices and Radiological Health, US Food and Drug Administration (FDA) commented that there is:

> *"currently insufficient scientific basis for concluding whether wireless communication technologies pose any health risk."*
>
> *"Little is known about the possible health effects of repeated or long-term exposures to low level RF of the sort emitted by such devices."*
>
> *"Some animal studies suggest the possibility for such low-level exposures to increase the risk of cancer…"*

Dr. Owen's comments are directed to users of cell phones, but the same questions are pertinent for long-term RF exposure to radiofrequency radiation for the larger broadcast transmissions of television, radio and wireless communications (Epidemiology Vol. 1, No. 2 March 2000 Commentary). The Food and Drug Administration signed an agreement (CRADA agreement) to provide funding for immediate research into RF health effects, to be funded by the Cellular Telephone Industry of America. The FDA no longer assures the safety of users. No completion date has been set.

XX. National Academy of Sciences - National Research Council

An Assessment of Non-Lethal Weapons Science and Technology by the Naval Studies Board, Division of Engineering and Physical Sciences (National Academies Press (2002) has produced a report that confirms the existence of non-thermal bioeffects from information transmitted by radiofrequency radiation at low intensities that cannot act by tissue heating (prepublication copy, page 2-13).

In this report, the section on Directed-Energy Non-Lethal Weapons it states that:

> *"The first radiofrequency non-lethal weapons, VMADS, is based on a biophysical susceptibility known empirically for decades. More in-depth health effects studies were launched only after the decision was made to develop that capability as a weapon. The heating action of RF signals is well understood and can be the basis for several additional directed-energy weapons. Leap-ahead non-lethal weapons technologies will probably be based on more subtle human/RF interactions in which the signal information within the RF exposure causes an effect other than simply heating: for example, stun, seizure, startle and decreased spontaneous activity. Recent developments in the technology are leading to ultrawideband, very high peak power and ultrashort signal capabilities, suggesting the the phase space to be explored for subtle, uyet potentially effective non-thermal biophysical susceptibilities is vast. Advances will require a dedicated effort to identify useful susceptibilities."*
>
> Page 2-13 of the prepublication report (emphasis added)

This admission by the Naval Studies Board confirms several critical issues with respect to non-thermal or low-intensity RF exposures. First, it confirms the existence of bioeffects from non-thermal exposure levels of RF. Second, it identifies that some of these non-thermal effects can be weaponized with bioeffects that are incontrovertibly adverse to health (stun, seizure, startle, decreased spontaneous activity). Third, it confirms that there has been knowledge for decades about the susceptibility of human beings to non-thermal levels of RF exposure. Fourth, it provides confirmation of the concept that radiofrequency interacts with humans based on the RF information content (signal information) rather than heating, so it can occur at subtle energy levels, not at high levels associated with tissue heating. Finally, the report indicates that a dedicated scientific research effort is needed to really understand and refine non-thermal RF as a weapon, but it is promising enough for continued federal funding.

XXI. The IEEE (United States)

IEEE ICES SCC-28 SC-4 Subcommittee (Radiofrequency/Microwave Radiation)

Members of the ICES SCC-28 SC-4 committee presented their views and justifications in a Supplement to the Bioelectromagnetics Journal (2003). It offers a window into the thinking that continues to support thermal-only risks, and on which the current United States IEEE recommendations have been made. The United States Federal Communications Commission (FCC) has historically based its federally-mandated public and occupational exposure standards on the recommendations of the IEEE.

Radiofrequency/Microwave Radiation

IEEE's original biological benchmark for setting human exposure standards (on which most contemporary human standards are based) is disruption of food-motivated learned behavior in subject animals. For RF, it was based on short, high intensity RF exposures that were sufficient to result in changes in animal behavior.

> *"The biological endpoint on which most contemporary standards are based is disruption of food- motivated learned behavior in subject animals. The threshold SAR for behavioral disruption has been found to reliably occur between 3 and 9 W/kg across a number of animal species and frequencies; a whole-body average SAR of 4 W/kg is considered the threshold below which adverse effects would not be expected. To ensure a margin of safety, the threshold SAR is reduced by a safety factor of 10 and 50 to yield basic restrictions of 0.4 W/kg and 0.08 W/kg for exposures in controlled (occupational) and uncontrolled (public) environments, respectively." (Osepchuk and Petersen, 2003).*

The development of public exposure standards for RF is thus based on acute, but not chronic exposures, fails to take into account intermittent exposures, fails to consider special impacts of pulsed RF and ELF-modulated RF, and fails to take into account bioeffects from long-term, low-intensity exposures that may lead to adverse health impacts over time.

XXII. BEMS Supplement 6 (Journal of the Bioelectromagnetics Society)

BEMS Supplement 6 was prepared in support of the IEEE SC-4 committee RF recommendations. In explaining and defending revised recommendations on RF limits contained within C.95.1, some key members took out space in Bioelectromagnetics (the Journal of the Bioelectromagnetic Society) to present papers ostensibly justifying a relaxation of the existing IEEE RF standards, rather than making the standards more conservative to reflect the emerging scientific evidence for both bioeffects and adverse health impacts.

Several clues are contained in the BEMS Supplement 6 to understand how the SC-4 IEEE C.95 revision working group and the ICES could arrive at a decision to not to recommend tighter limits on RF exposure. Not one but two definitions of "adverse effect" are described, one by Osepchuk/Petersen (2003) and another by the working group itself (D'Andrea et al, 2003). Both set a very high bar for demonstration of proof, and both are ignored in the final recommendations by the SC-4 Subcommittee.

Second, many of the findings presented in the papers by individual authors in the BEMS Supplement 6 do report that RF exposures are linked to bioeffects and to adverse effects; but these findings are evidently ignored or dismissed by the SC-4 Subcommittee, ICES and by the eventual adoption of these recommendations by the full IEEE membership (in 2006). Even with a very high bar of evidence set by the SC-4 Subcommittee (and two somewhat conflicting definitions of adverse effect against which all scientific papers were reviewed and analyzed); there is clear sign that the "deal was done' regardless of even some of the key Subcommittee member findings reporting such effects at exposure levels below the existing limits.* sidebar

The SC-4 Subcommittee has developed a new and highly limited definition on RF effects, adverse effects and hazards that is counter to the WHO Constitution Principle on Health. The definition as presented by D'Andrea et al (2003, page S138) is based on the SC-4 IEEE C.95 revision working group definition of adverse effect:

"An adverse effect is a biological effect characterized by a harmful change in health. For example, such changes can include organic disease, impaired mental function, behavioral disfunction, reduced longevity, and defective or deficient reproduction. Adverse effects do not include: biological effects without detrimental health effect, changes in subjective feelings of well-being that are a result of anxiety about RF effects or impacts of RF infrastructure that are not related to RF emissions, or indirect effects caused by electromagnetic interference with electronic devices. An adverse effects exposure level is the condition or set of conditions under which an electric, magnetic or electromagnetic field has an adverse effect."

Further, the working group extended its definition to include that of Michaelson and Lin (1987) which states:

"If an effect is of such an intense nature that it compromises the individual's ability to function properly or overcomes the recovery capability of the individual, then the 'effect' may be considered a hazard. In any discussion of the potential for 'biological effects' from exposure to electromagnetic energies we must first determine whether any 'effect' can be shown; and then determine whether such an observed 'effect' is hazardous."

The definition of adverse effect according to Osepchuk and Petersen (2003) reported in the same BEMS Supplement 6 is:

"An adverse biological response is considered any biochemical change, functional impairment, or pathological lesion that could impair performance and reduce the ability of an organism to respond to additional challenge. Adverse biological responses should be distinguished from biological responses in general, which could be adaptive or compensatory, harmful, or beneficial. "

In contrast, the World Health Organization draft framework has accepted definitions of bioeffect, adverse health effect and hazard (WHO EMF Program Framework for Developing EMF Standards, Draft, October 2003). These definitions are not subject to the whim of organizations preparing public exposure standard recommendations. The WHO definition states that:

"(A)nnoyance or discomforts caused by EMF exposure may not be pathological per se, but, if substantiated, can affect the physical and mental well-being of a person and the resultant effect may be considered as an adverse health effect. A health effect is thus defined as a biological effect that is detrimental to health or well-being. According to the WHO Constitution, health is a state of complete physical, mental, and social well-being and not merely the absence of disease or infirmity."

The SC-4 definitions require proof that RF has caused organic disease or other cited effects that qualify. The burden of proof is ultimately shifted to the public, that bears the burden of unacknowledged health effects and diseases, where the only remedy is proof of illness over a large population of affected individuals, over a significant amount of time, and finally, delays until revisions of the standards can be implemented. The results of studies and reviews in the BEMS Supplement 6 already acknowledge the existence of bioeffects and adverse effects that occur at non-thermal exposure levels (below current FCC and ICNIRP standards that are supposedly protective of public health. However, they go on to ignore their own findings, and posit in advance that adverse effects seen today will, even with chronic exposure, not conclusively reveal disease or dysfunction tomorrow at exposure levels below the existing standards.

Sidebar: Quotes from BEMS Supplement 6

a) Studies and reviews where bioeffects likely to lead to adverse health effects with chronic exposure are reported;
b) adverse effects which are already documented;
c) studies where non-thermal RF effects are reported and unexplained;
d) effects are occurring below current exposure limits, and
e) conclusions by authors they cannot draw conclusions about hazards to human health

These quotes appear in articles presented by the IEEE SC-4 Subcommittee in BEMS Supplement 6. Despite these acknowledged gaps in information, lack of consistency in studies, abundant conflicting evidence documenting low level RF effects that can resulting serious adverse health impacts (DNA damage, cognitive impairment, neurological deficits, cancer, etc), and other clear instances of denial of ability to predict human health outcomes, the IEEE SC-4 Subcommittee has proposed recommendations to relax the existing limits.

XXIII. Proceedings of the NATO Advanced Research Workshop – Mechanisms of the Biological Effect on Extra High Power Pulses (EHPP) and UNESCO/WHO/IUPAB Seminar "Molecular and Cellular Mechanisms of Biological Effects of EMF" held March 2005, Yerevan, Armenia.

The proceedings conclude that "*the authors agreed with one main conclusion from these meeting(s): that in the future worldwide harmonization of standards have to be based on biological responses, rather than computed values*". The authors included 47 scientists, engineers, physicians and policy makers from 21 countries from Europe, North and South America, and Asia.

> *"The ICNIRP Guidelines for radiofrequency electromagnetic exposure are based only on thermal effects, and completely neglects the possibility of non-thermal effect."*
>
> *"The guidelines of the International Commission on Non-Ionizing Radiation Protection (ICNIRP) specify the quantative characteristics of EMF used to specify the basic restrictions are current density, specific absorption rate (SAR) and power density, i.e., the energetic characteristics of EMF. However, experimental data on energy-dependency of biological effects by EMF have shown that the SAR approach, very often, neither adequately describes or explains the real value of EMF-induced biological effects on cells and organisms, for at least two reasons: a) the non-linear character of EMF-induced bioeffects due to the existence of amplitude, frequency and 'exposure time-windows' and b) EMF-induced bioeffects significantly depend on physical and chemical composition of the surrounding medium."* (Preface pages XI – XIII).

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SECTION 5

Evidence For Effects On Gene And Protein Expression

(Transcriptomic and Proteomic Research)

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I. INTRODUCTION

Daily exposure to electromagnetic fields (EMF), including extremely low frequency magnetic fields (ELF MF) and radiofrequency (RF) EMF, in the environment has raised public concerns about whether they have harmful consequences on human health. Several epidemiological studies suggest that exposure to EMF might associate with an elevated risk of cancer and other diseases in humans (reviewed in [Feychting et al., 2005]). To explain and/or support epidemiological observations, many laboratory studies have been conducted, but the results were controversial and no clear conclusion could be drawn to assess EMF health risk.

It is reasoned that one of the priorities in EMF research is to elucidate the biological effects of EMF exposure and the underlining mechanisms of action. Gene and protein are key players in organisms, and it has been assumed that any biological impact of EMF must be mediated by alterations in gene and protein expression [Phillips et al., 1992; Wei et al., 1990]. For example, heat shock protein, c-myc, and c-jun have been identified as EMF responsive genes and/or proteins in certain biological systems. In order to reveal the global effects of EMF on gene and protein expression, transcriptomics and proteomics, as high-throughput screening techniques (HTSTs), were eventually employed in EMF research with an intention to screen potential EMF-responsive genes and/or proteins without any bias. In 2005, WHO organized a Workshop on Application of Proteomics and Transcriptomics in EMF Research in Helsinki, Finland to discuss the related problems and solutions in this field [Leszczynski 2006; Leszczynski and Meltz 2006]. Later the journal Proteomics published a special issue devoted to the application of proteomics and transcriptomics to EMF research. This review aims to summarize the current research progress and discuss the applicability of HTSTs in the field.

II. ELF MF

II A. TRANSCRIPTOMICS

Binninger and Ungvichian firstly measured purified mRNA levels of total RNA from MF- and sham-exposed yeast cells and reported that the levels of a significant proportion of mRNAs were altered in response to continuous exposure to 20 μT 60 Hz MF over a period of approximately 15 cell generations (24 h) [Binninger and Ungvichian 1997]. Unfortunately, no reproducible genes (polypetides) were identified in this study although the authors consistently found different proportions of transcripts whose abundances were altered in all four replication experiments.

Wu *et al.* have applied differential display reverse transcriptase–polymerase chain reaction (DD-RT-PCR) and Northern blotting to screen MF-responsive gene in Daudi cells. The cells were exposed to 0.8 mT of 50 Hz MF for 24 h. The authors screened out two candidate genes in Daudi cells and one was identified as a MF-responsive gene *ceramide glucosyltransferase*. They further found time-dependent changes in the transcription of *ceramide glucosyltransferase* induced by 0.8 mT MF [Wu et al., 2000].

With the help of DD-RT-PCR, Olivares-Banuelos *et al* reported that exposure to 0.7 mT 60 Hz MF for 7 days，4 h a day (2 h in the morning and 2 h in the afternoon), changed the global transcription profile of chromaffin cells. Eight RT-PCR products which correspond to six genes were identified, including *phosphoglucomutase*-1, *neurofibromatosis-2 interacting protein*, *microtubule associated protein*-2, *thiamine pyrophosphokinase*, and two hypothetical proteins (RNOR02022103 and ROR01044577). In addition, the authors found that presumed regulatory regions of these genes contained CTCT-clusters [Olivares-Banuelos et al., 2004], which has been identified as an electromagnetic field-responsive DNA element regulating gene expression [Goodman and Blank 2002].

Balcer-Kubiczek *et al.* have applied the two-gel cDNA library screening method (BIGEL) to screen MF-responsive genes, in which the gel arrays contained a total of

960 cDNAs selected at random from the cDNA library. The HL 60 cells were exposed to 2 mT of 60 Hz square wave MF for 24 h. Four candidate genes were shown responsive to the MF exposure, but could not be confirmed by following Northern analysis. Furthermore, the authors found that these four candidates and another four selected genes (*MYC*, *HSP70*, *RAN* and *SOD1*) did not react to either square wave or sine wave 60 Hz MF at 2 mT for 24 h [Balcer-Kubiczek et al., 2000]. However, the cellular responses to square wave and sine wave 60 Hz MF might be different. In order to systematically evaluate the effect of 60 Hz MF on gene expression in HL 60 cells, it is necessary for the authors to screen 60 Hz sine wave MF responsive candidate genes in HL 60 cells with BIGEL method as well, and then, perform validation with Northern blotting for these candidates.

Using cDNA arrays containing 588 cancer-related genes, Loberg *et al.* analyzed gene expression in normal (HME) and transformed (HBL-100) human mammary epithelial cells and human promyelocytic leukemia (HL60) cells after exposure to 60 Hz MF at intensity of 0.01 or 1.0 mT for 24 h. The authors reported that several genes were identified in MF-exposed cells whose expressions were increased by at least two folds or decreased by 50% or more, but no gene was found to be differentially expressed in each of three independent exposures for any cell type, and no relationship between exposure intensity and differential gene expression was found [Loberg et al., 2000].

In order to obtain a more global evaluation, genome-wide microarray screening methods were applied to identify genes responding to ELF MF in certain types of cells. By application of cDNA microarray, Nakasono *et al.* have investigated the effect of 50 Hz MF below 300 mT on gene expression in yeast. The authors reported that several genes were found differentially expressed in yeast cells with medium to low confidence level (CL) after exposure to 10, 150 and 300 mT for 24 h. Among these genes, seven showed a dose-response relationship in the normalized ratio data and three genes showed a reproducible change for all three intensities. They also proposed that these genes should be re-examined by methods with greater sensitivity or by quantitative methods, such as real-time PCR. On the other hand, no high-confidence expression changes were observed for genes that are involved in heat-shock response, DNA repair, respiration, protein synthesis, or cell cycle. Thus, they concluded that 50 Hz MF up to 300 mT did not appear to affect gene expression linked to either defined cell processes stated above or unknown cell responses in investigated model eukaryotic cells [Nakasono et al., 2003]. Unfortunately, only single experiment for array analysis was performed in this study.

Recently, a similar study was conducted by Luceri *et al.* to investigate the global gene response to 50 Hz MF in human lymphocytes and yeast cells. These two types of cells were exposed to MF at intensity of 100 μT, 10 μT and 1 μT for 18 h. As a result, in lymphocytes, one gene was found down-regulated at 100 μT, one down-regulated gene and two up-regulated genes were screened out at 10 μT, and no gene was detected changed at 1 μT. As to the yeast cells, the results showed 2, 15 and 2 genes as differentially expressed (mainly down-regulated) after exposure to 100, 10 and 1 μT, respectively, in which SPS100 gene was consistently up-regulated after exposure to 50 Hz MF at all three intensities. But no genes were found differentially expressed when the authors analyzed the data by other statistical methods. Thus, the authors concluded that 50 Hz MF did not affect gene expression in these two types of cells and the variations of a few genes mentioned above could be due to experimental noise [Luceri et al., 2005]. However, it is necessary to examine the candidates, especially the SPS100 gene, to validate whether they were real “un-responsive” genes.

In Henderson’s report, human umbilical vein endothelial cells (HUVEC) were exposed to various patterns and intensities of 50 Hz MF, including continuous exposure at a two intensities (10 and 700 μT), intermittent exposure (60 min on/ 30 min off) at a single intensity (700 μT), and continuous exposure to a variable-intensity fields (10-30 μT). The transcriptional response of the cells was investigated using oligonucleotide microarrays containing up to 30, 000 unique features. Although different genes were identified where their expressions appeared to be affected by exposure to MF in individual experiments, none of these genes were regulated in the same manner in subsequent repetition experiments [Henderson et al., 2006].

Antonini *et al* reported that intermittent exposure (5 min on/5 min off) to 50 Hz MF at flux densities of 2 mT for 16 h could change gene expression in human neuroblastoma cell line SH-SY5Y by application of whole-genome Human Unigene RZPD-2 cDNA array which contains about 75, 000 cDNA clones. Several genes were found down- or up-regulated at least five-fold after ELF MF exposure and the authors concluded that SH-SY5Y cells were sensitive to ELF MF [Antonini et al., 2006]. However, no reports indicated that these differentially expressed genes were confirmed by other methods.

Lupke *et al* investigated the effect of ELF MF on gene expression profiling in human umbilical cord blood-derived monocytes using the same Unigene RZPD-2. The results indicated that 0.1 mT 50 Hz MF exposure for 45 minutes altered the expressions of 986 genes involved in metabolism, cellular physiological processes, signal transduction, and immune response, among them, five genes were significantly regulated. Furthermore, the authors analyzed several genes by real-time RT-PCR and one ELF MF candidate responsive gene IL15RA was confirmed. However, this study only did single array analysis for pooling sample from 78 donors and two independent real-time RT-PCR analyses for samples from 5 and 6 different donors. The authors did not report the examinations of other candidates with real-time RT-PCR analysis [Lupke et al., 2006].

II B. PROTEOMICS

Nakasono *et al.* has investigated the effects of protein expression in model system such as *Escherichia coli* and *Saccharomyces cerevisiae* using two dimensional gels electrophoresis (2-DE) method. When the bacterial cells were exposed to each MF at 5-100 Hz under aerobic conditions (6.5 h) or at 50 Hz under anaerobic conditions (16 h) at the maximum intensity (7.8 to 14 mT), no reproducible changes were observed in the 2D gels. However, the stress-sensitive proteins did respond to most stress factors, including temperature change, chemical compounds, heavy metals, and nutrients. The authors concluded that the high-intensity ELF MF (14 mT at power frequency) did not act as a general stress factor [Nakasono and Saiki 2000]. When using *Saccharomyces cerevisiae* as a model system, Nakasono *et al.* reported that no reproducible changes in the 2D gels were observed in yeast cells after exposure to 50 Hz MF at the intensity up to 300 mT for 24 h [Nakasono et al., 2003]. In this study, only three sets of gels from three independent experiments were analyzed.

Li *et al.* have performed a proteomics approach to investigate the changes of protein expression profile induced by ELF MF in human breast cancer cell line MCF-7. With help of 2-DE and data analysis on nine gels for each group, 44 differentially expressed protein spots were screened in MCF-7 cells after exposure to 0.4 mT 50 Hz MF for 24 h. Three proteins were identified by LC-IT Tandem MS as RNA binding protein regulatory subunit, proteasome subunit beta type 7 precursor, and translationally controlled tumor protein, respectively [Li et al 2005]. Further investigations, such as Western blotting, are required to confirm these ELF responsive candidate proteins.

Using 2-D Fluorescence Difference Gel Electrophoresis (2-D DIGE) technology and MS in a blind study, Sinclair *et al* have investigated the effects of ELF MF on the proteomes of wild type *Schizosaccharomyces pombe* and a Sty1p deletion mutant which displays increased sensitivity to a variety of cellular stresses. The yeast cells were exposed to 50 Hz EMF at field strength of 1 mT for 60 min. While this study identified a number of protein isoforms that displayed significant differential expressions across experimental conditions, there was no correlation between their patterns of expression and the ELF MF exposure regimen. The authors concluded that there were no significant effects of ELF MF on the yeast proteome at the sensitivity afforded by 2D-DIGE. They hypothesized that the proteins identified in the experiments must be sensitive to subtle changes in culture and/or handling conditions. Based on their experience, they suggested to the community that the interpretation of proteomic data in a biological context should be treated with caution [Sinclair et al., 2006].

II C. SUMMARY

Generally, recent studies on global gene and protein expression responding to ELF MF have been conducted in different biological systems by applications of HTSTs. Only a few studies reported to identify ELF MF responsive genes successfully. For example Wu *et al.* identified *ceramide glucosyltransferase* as a MF-responsive gene in Daudi cells [Wu et al., 2000] and Olivares-Banuelos *et al.* identified six ELF MF genes in chromaffin cells [Olivares-Banuelos et al., 2004] with the help of DD-RT-PCR and Northern blotting analysis; by combining cDNA array analysis with real-time RT-PCR confimation, Lupke *et al.* identified IL15RA as ELF MF responsive genes in human monocytes [Lupke et al., 2006]. Although many transcriptome and proteome analysis showed that ELF MF exposure could change gene and/or protein expression in certain cell types [Antonini et al., 2006; Binninger and Ungvichian 1997; Li et al., 2005], there are lack of confirmation to determine if they are real ELF MF responsive genes or proteins. Therefore, it is a priority to conduct confirmation experiments to demonstrate the author’s findings.

As to those negative reports, few or no genes and proteins were found significantly changed according to their statistical analysis and screening standards. But these few genes and proteins were neither reproducible [Henderson et al., 2006; Nakasono et al., 2003; Sinclair et al., 2006]nor confirmed by other methods [Balcer-Kubiczek et al., 2000], and the changes were not related to ELF MF exposure [Loberg et al., 2000; Luceri et al., 2005; Nakasono et al., 2003]. Therefore, these studies are also needed to be replicated or verified.

III. RF EMF

III A . TRANSCRIPTOMICS

In an initial study utilizing membrane-based cDNA microarray, Harvey and French studied the effects of 864.3 MHz (CW) on HMC-1 human monocytes. The exposure was carefully controlled and averaged at an SAR of 7 W/kg, almost double the exposure level of established adverse effects. Three 20 min exposures were performed at 4-h intervals daily for 7 days. cDNA microarray analyses revealed consistent alterations in steady-state mRNA levels of 3 of the 558 genes represented on the membranes including one proto-oncogene *c-kit* (increased), one apoptosis-associated gene *DAD-1* (decreased) and one potential tumor suppressor gene *NDPK* (decreased) [Harvey and French 1999]. However, there were considerable variabilities between the two experiments reported and the fold change of each differentially expressed gene was small (< 1.5 folds). Meanwhile, the authors did not use other methods to confirm the results.

Pacini *et al.* investigated the effect of gene expression in human skin fibroblasts by using cDNA arrays including 82 genes, and reported that exposure to GSM 902.4 MHz RF EMF at an average SAR of 0.6 W/kg for 1 h increased the expression of 14 genes which function in mitogenic signal transduction, cell growth and apoptosis controlling. The authors further demonstrated a significant increase in DNA synthesis and intracellular mitogenic second messenger formation which were matched the high expression of MAP kinase family genes [Pacini et al., 2002]. The authors suggested that the RF EMF exposure has significant biological effects on human skin fibroblasts.

However, only one experiment was performed in array analysis and no more experiment was made by the authors to confirm the array analysis result.

With help of cDNA microarray, Leszczynski *et al*. reported that exposure to GSM 900 MHz RF EMF at an average SAR of 2.4 W/kg for 1 h changed expression of 3600 genes, including down-regulated genes involved in forming the Fas/TNFa apoptotic pathway in human endothelial cell line EA.hy926 [Leszczynski et al., 2004]. The authors performed three separate experiments in array analysis, but no confirmation experiments were conducted to validate the array analysis result. Recently, Leszczynski group compared the global gene response of two human endothelial cells, EA.hy926 and its variant EA.hy926v1 to RF EMF and reported that the same genes were differently affected by the exposure to GSM 900 MHz RF EMF at an average SAR of 2.8 W/kg for 1 h in each of the cell lines [Nylund and Leszczynski 2006]. Similarly, no reports indicated that the differentially expressed genes in this study were confirmed by other methods.

Lee *et al*. used the serial analysis of gene expression (SAGE) method to measure the RF EMF effect on genome scale gene expression in HL 60 cells. The cells were exposed to 2.45 GHz RF EMF at an average SAR of 10 W/kg for 2 h and 6 h. The authors observed that 221 genes and 759 genes altered their expression after 2 h exposure and 6 h exposure respectively. Functional classification of the affected genes revealed that apoptosis-related genes were among the up-regulated ones and the cell cycle genes among the down-regulated ones, but no significant increase in the expression of heat shock genes were found [Lee et al., 2005]. However, the SAGE experiment was repeated only once and only one control with 2 h sham exposure was used. No confirmation experiment was reported to validate these differentially expressed genes.

Huang *et al*. investigated the effect of 1763 MHz RF EMF on gene expression in Jurkat cells by Applied Biosystems 1700 full genome expression microarray. The authors found that 68 genes were differentially expressed in the cells after exposure to RF EMF at SAR of 10 W/kg for 1 h and harvested immediately or after 5 h [Huang et al., 2006]. The authors repeated sets of experiment five times to collect biological triplicates in every sample but the differentially expressed genes were not confirmed by other methods.

Whitehead *et al.* have performed *in vitro* experiments with C3H 10T(1/2) mouse cells to determine whether Frequency Division Multiple Access (FDMA) or Code Division Multiple Access (CDMA) modulated RF radiations can induce changes in gene expression using the Affymetrix U74Av2 GeneChip. The GenesChip data showed the number of probe sets with an expression change greater than 1.3-fold was less than or equal to the expected number of false positives in C3H 10T(1/2) mouse cells after 835.62 MHz FDMA or 847.74 MHz CDMA modulated RF EMF exposure at SAR of 5 W/kg for 24 h. The authors concluded that the 24 h exposures to FDMA or CDMA RF radiation at 5 W/kg had no statistically significant effect on gene expression [Whitehead et al., 2006a; Whitehead et al., 2006b]. However, the authors did not demonstrate that these differentially expressed genes were real “false positive” with other methods.

In Gurisik’s report, human neuroblastoma cells (SK-N-SH) were exposed to GSM 900 MHz RF signal at SAR of 0.2 W/kg for 2 h and recovered without field for 2 h post-exposure. Gene expression were examined by Affymetrix Human Focus Gene Arrays including 8400 genes and followed by real-time RT-PCR of the genes of interest. Only six genes were found to be slightly down-regulated in response to RF exposure comparing with mock-exposed cells. Furthermore, these genes can not be confirmed by real-time RT-PCR analysis. Thus, the authors concluded that the RF EMF exposure applied in this study could not change gene expression in SK-N-SH cells [Gurisik et al., 2006]. However, the array analysis experiment was repeated only once and only one array for exposure or sham exposure group.

Qutob *et al* have assessed the ability of exposure to a 1.9 GHz pulse-modulated RF field to affect global gene expression in U87MG glioblastoma cells by application of Agilent Human 1A (v1) oligonucleotide 22K microarray slides. The U87MG cells were exposed to 1.9 GHz pulse-modulated (50 Hz, 1/3 duty cycle) RF field at an average SAR of 0.1, 1.0 and 10.0 W/kg for 4 hours, and incubated for an additional 6 hours. The authors found no evidence that exposure to RF fields under different exposure conditions can affect gene expression in cultured U87MG cells. In this paper, the authors performed five experiments, each containing a single replicate and some of genes were confirmed as real "un-effected genes" [Qutob et al., 2006].

Zeng *et al*. have investigated gene expression profile in MCF-7 after exposing to GSM 1800 MHz RF EMF using Affymetrix Genechip U133A. The result showed that no gene with 100% consistency change were found in MCF-7 cells after intermittent exposure (5 min on/ 10 min off) to RF EMF at an average SAR of 2.0 W/kg for 24 h while five genes with 100% consistency change were found in MCF-7 at same exposure conditions but at SAR of 3.5 W/kg. However, these five differentially transcribed genes could not be further confirmed by real-time RT-PCR assay. Thus, this study did not provide evidence that RF EMF exposure can produce distinct effects on gene expression in the MCF-7 cells [Zeng et al., 2006].

Remondini *et al*. have investigated the effect of RF EMF on gene expression profile in six different cell lines or primary cells, and found various types of cell reacted differently in RF EMF exposure). RF EMF exposure changed gene expression in 900 MHz-exposed EA.hy926 endothelial cells (22 up-regulations, ten down-regulations), 900 MHz-exposed U937 lymphoblastoma cells (32 up-regulations, two down-regulations), and 1800 MHz-exposed HL-60 leukemia cells (11 up-regulations, one down-regulation) while NB69 neuroblastoma cells, T-lymphocytes, and CHME5 microglial cells did not show significant changes in gene expression. The authors concluded that there were alterations in gene expression in some human cells types exposed to RF-EMF but these chenges depended on the type of cells and RF-EMF signal [Remondini et al., 2006]. However, these RF responsive candidate genes in different types of cells were not confirmed yet.

Very recently, Zhao *et al*. have investigated the effects of RF EMF on gene expression of *in vitro* cultured rat neuron with Affymetrix Rat Neurobiology U34 array. Among 1200 candidate genes, 24 up-regulted genes and 10 down-regulated genes were identified after 24-h intermittent exposure (5 min on/ 10 min off) at an average SAR of 2.0 W/kg, which are associated with multiple cellular functions. The changes of most of genes were successfully validated by real-time RT-PCR, including genes involved in cytoskeleton, signal transduction pathway, metabolism [Zhao et al., 2007].

Belyaev et al. analyzed gene expression profile in RF exposed animals. Rats were exposed or sham exposed to GSM 915 MHz at whole body average SAR of 0.4 mW/g for 2 h and total RNA was extracted from cerebellum. Gene expression profiles were obtained by Affymetrix U34 GeneChips representing 8800 rat genes and analyzed with the Affymetrix Microarray Suite (MAS) 5.0 software. The results showed that 11 genes were up-regulated in a range of 1.34-2.74 folds and one gene was down-regulated 0.48-fold. The induced genes encode proteins with diverse functions including neurotransmitter regulation, blood-brain barrier (BBB), and melatonin production [Belyaev et al., 2006]. In this study, triplicate arrays were applied for three exposed samples or three sham exposed samples. But the differentially expressed genes were not confirmed by other methods.

III B . PROTEOMICS

Leszczynski *et al*. have provided perhaps some of the most relevant *in vitro* data by studying the effects of GSM 900 MHz RF EMF exposure [Leszczynski et al., 2002; Nylund and Leszczynski 2004; Nylund and Leszczynski 2006]. Firstly, the EA.hy926 cells were exposed to RF EMF at SAR of 2.0 W/kg over a one-hour period and the data indicated the RF exposure changed protein expression at a proteome scale, and up-regulated the level of HSP 27 protein and induced its hyper-phosphorylation. The activation of p38 mitogen activated kinase (MAPK) was partially responsible for the phosphorylation of the HSP. They confirmed HSP27 protein expression, phosphorylation and cellular distribution by independent protein analytical techniques including western blotting and indirect immunofluorescence [Leszczynski et al., 2002]. Secondly, the group screened 38 proteins with statistically significantly altered expression in the same cell line after GSM 900 MHz exposure at SAR of 2.4 W/kg for 1 h. An isoform of vimentin was confirmed as a responsive protein by Western blotting and indirect immunofluorescence. The authors concluded that the cytoskeleton might be one of the mobile phone radiation-responding cytoplasmic structures [Nylund and Leszczynski 2004]. Furthermore, they compared *in vitro* response to GSM 900 MHz RF EMF in EA.hy926 with its variant EA.hy926v1 by examination of protein expression using 2-DE. The results showed protein expression profiles were altered in both examined cell lines after RF EMF exposure. However, the affected proteins were differently in each of the cell lines, 38 and 45 differentially expressed proteins were found in EA.hy926 and EA.hy926v1 respectively. Several differentially expressed proteins in EA.hy926 cells were confirmed by other methods, but no differentially expressed protein in EA.hy926v1 cells was confirmed. Base on the transcriptome and proteome analysis data, the authors concluded that the response might be genome- and proteome-dependent [Nylund and Leszczynski 2006]. One thing should be mentioned that all the 2-DE analyses in Leszczynski group reports were replicated ten times.

Zeng *et al.* systematically explored the effects of 1800 MHz RF EMF on protein expression in MCF-7 cells by 2-DE, and revealed that a few but different proteins were differentially expressed under continuous or intermittent RF EMF exposure at SAR of 3.5 W/kg for 24 h or less, implying that the observed effects might have occurred by chance. By combination with the transcriptomics analysis data, this study did not provide convincing evidence that RF EMF exposure could produce distinct effects on gene and protein expression in the MCF-7 cells. The authors supposed that the MCF-7 cells may be less sensitive to RF EMF exposure [Zeng et al., 2006]. However, in this study, only triplicate gels were performed in each exposure condition experiment.

III C . SUMMARY

The effects of RF EMF on global gene and protein expression have been investigated in different biological systems, and most of studies were focused on the mobile phone utilization frequency (800-2000 MHz) at relative low exposure density (average SAR near 2.0 W/kg). Some studies reported negative results of RF EMF exposure on gene expression. For example, Whitehead *et al.* did not find differentially expressed genes in RF exposed C3H 10T(1/2) mouse cells [Whitehead et al., 2006a; Whitehead et al., 2006b]. Remondini *et al.* reported that NB69 cells, T lymphocytes, and CHME5 cells did not show significant changes in gene expression after RF EMF exposure [Remondini et al., 2006]. In Gurisik *et al.* [Gurisik et al., 2006]and Zeng *et al.* [Zeng et al., 2006]study, although they screened out several RF EMF-responsive candidate genes, they could not confirm these genes by real-time RT-PCR method.

Meanwhile, several groups claimed that RF EMF exposure can change gene and protein expression profile in certain types of cells and identified certain EMF responsive genes and proteins. Only one report found RF EMF exposure changed gene expression profile in neurons and most of changed genes were confirmed by real-time RT-PCR [Zhao et al 2007]. As to proteome analysis, only two groups have analyzed protein expression by proteomic approaches, including 2-DE and Mass Spectrum. Zeng *et al.* systematically explored the effects of 1800 MHz RF EMF on protein expression in MCF-7 cells by 2-DE, and revealed that a few but different proteins were differentially expressed under different exposure conditions, implying that the observed effects might have occurred by chance [Zeng et al., 2006]. However, in this study, only triplicate gels were performed in each exposure condition experiment. In contrast, Leszczynski group identified two RF EMF responsive proteins in EA.hy926 cells, i.e. HSP27 [Leszczynski et al., 2002] and vimentin [Leszczynski et al., 2004] with help of 2-DE and MS analysis. This group further confirmed the expression and cellular distribution of HSP27 and vimentin in RF exposed EA.hey926 cells by other methods including Western blotting and indirect immunofluorescence staining. Furthermore, they reported the changes of these RF EMF molecular targets had down-stream impact on cell physiology [Leszczynski et al., 2002; Leszczynski et al., 2004].

Generally, it seems that the response of a cell to RF EMF exposure depends on exposure condition, cell type, and/or the cell's genome- and proteome [[Remondini et al., 2006; Nylund and Leszczynski 2006].

IV. Overall Conclusion

Based on current available literature, it is justified to conclude that EMF exposure can change gene and/or protein expression in certain types of cells, even at intensities lower than ICNIRP recommended values. However, the biological consequences of most of the changed genes/proteins are still unclear, and need to be further explored. Thus, it is not the time point yet to assess the health impact of EMF based on the gene and protein expression data. The IEEE and WHO data bases do not include the majority of ELF studies; they do include the majority of the RF studies.

Currently, controversial data exist in the literature. The EMF research community should pay equal attention to the negative reports as to the positive ones. Not only the positive findings need to be replicated, all the negative ones are also needed to be validated.

It is noteworthy that low intensity EMF is a weak physical stimulus for a cell or organism, and high throughput screening techniques (HTSTs) would sacrifice its sensitivity to ensure its high throughput. It has been recognized there is methodological defects while analyzing weak effect with HTSTs, such as reproducibility and variability.

Thus, more experimental replications are needed to reduce the ratio of noise over signal. Meanwhile, confirmation study must be included to assure the validity of the data.

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 5

Evidence for EMF Transcriptomics and Proteomics Research 2007-2012

2012 Supplement

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I. INTRODUCTION

Daily exposure levels for non-ionizing electromagnetic radiation (NI-EMR) have significantly increased in the last few decades for human populations, and for wildlife, plants, and other living creatures on earth. NI-EMR includes a wide range of frequencies, as low as extremely low frequencies (ELF) magnetic fields deriving from the power lines up to microwave radiofrequencies (MW-RF). Within this range are FM and TV broadcast stations, wireless technology devices (mobile phones and masts, cordless phones, Wi-Fi routers and units).

The exposure to any of these frequencies individually, or in combination, raises concern about potentially harmful effects and is the subject of intensive scientific studies around the world. Such studies include epidemiological, clinical, *in vivo* and*in vitro* studies. The pace of scientific study accelerated after 2010, when the World Health Organization following the ELF agenda of 2007 (WHO, 2007), announced the implementation of the International EMF Project's RF Research Agenda as a "*research topic for measurement surveys to characterize population exposures from all radio frequency (RF) sources with a particular emphasis on new wireless technologies*" (WHO, 2010). The IARC (International Agency for Research on Cancer) under the auspices of the WHO classified RFR as a Possible Human Carcinogen (Group 2B) on 2011 (Baan et al., 2011).

The studies published so far have utilized various model systems and approaches but not in a coordinated manner, although there have been international efforts (i.e., INTERPHONE Final Study; Cardis et al., 2011).

As reviewed by Vlaanderen et al. (2009), OMICS technologies are relatively new biomarker discovery tools that can be applied to study large sets of biological molecules. (The English-language neologism omics informally refers to a field of study in biology ending in *-omics*, such as genomics, proteomics or metabolomics). Their applications in EMF and RFR research have become feasible in recent years due to a spectacular increase in the sensitivity, resolution and throughput of OMICS-based assays (Vlaanderen et al., 2009).

.Although, the number of OMIC techniques is ever expanding, the five most developed OMICS technologies are genotyping, transcriptomics, epigenomics, proteomics and metabolomics.

A number of reports have dealt with possible changes on gene/protein expression, either at an individual gene/protein level or using the high throughput "omics" approaches (T & P -transcriptomics and proteomics respectively) (for reviews see Xu & Chen, 2007; Blankenburg et al., 2009; McNamee & Chauhan, 2009; Mevissen M., 2011; Leszczynski et al., 2012). These T & P approaches have gained ground in the investigation of the possible EMF effects the last decade (Blankenburg et al., 2009), since they can screen the whole genome or proteome and may contribute on the elucidation of EMF mechanisms of action.

Following the work of Xu and Chen who gathered all studies on EMF research using T & P high throughput approaches up to 2006 in the BioInitiative Report (Xu & Chen, 2007), this supplemental chapter on Transcriptomics and Proteomics updates newly published work since that initial review in 2007.

II. EXREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS (ELF-EMFS)

A. Transcriptomics

As explicitly described by M. Mevissen (2011), gene expression profiling is the identification and characterization of the mixture of mRNA that is present in a specific sample. Both the presence of specific forms of mRNA and the levels in which these forms occur are parameters that provide information on gene expression. A gene expression profile provides a quantitative overview of the mRNA transcripts that were present in a sample at the time of collection. Therefore, gene expression profiling can be used to determine which genes are differently expressed as a result of changes in environmental conditions. DNA Microarrays represent an innovative and comprehensive technology that allows researchers to assess the expression level of thousands of genes in a high-throughput fashion and has been exploited in EMF research studies.

Schwenzer et al. (2007) reported effects of static magnetic field on genome expression. Specifically, the researchers evaluated the influence of magnetic resonance imaging (MRI) on gene expression in embryonic human lung fibroblasts (Hel 299). The cells were exposed to the static magnetic field and to a turbo spin-echo sequence of an MR scanner at 3.0 Tesla. An MR group (exposed) and a control group (sham-exposed) were set up using a special MR-compatible incubation system. The exposure time was two hours. Gene expression profiles were studied using a complementary deoxyribonucleic acid (cDNA) microarray containing 498 known genes involved in transcription, intracellular transport, structure/junction/adhesion or extracellular matrix, signalling, host defence, energetics, metabolism, cell shape, and death. No changes in gene expression were found in either group (exposed or sham-exposed cells) at the end of a two-hour exposure for any of the 498 tested protein genes. The results showed that MRI had no influence on protein–gene expression in eugenic human lung cells in this study.

The same year, Walther et al. (2007) analyzed the effects of BEMER type (combination of electromagnetic field and light therapy) electromagnetic field (BTEMF) on gene expression in human mesenchymal stem cells and chondrocytes. Primary mesenchymal stem cells from bone marrow and the chondrocyte cell line C28I2 were stimulated 5 times at 12-h intervals for 8 min each with BTEMF. RNA from treated and control cells was analyzed for gene expression using the affymetrix chip HG-U133A. A limited number of regulated gene products from both cell types, which control cell metabolism and cell matrix structure, was mainly affected. There was no increased expression though of cancer-related genes. RT-PCR analysis of selected transcripts partly confirmed array data. Results indicate that BTEMF in human mesenchymal stem cells and chondrocytes provide the first indications. A limitation of this study is the single array analysis which was performed. Therefore, as stated by the authors, the results should be regarded as a first hint on BTEMF effects on these cellular systems. Nevertheless, their findings indicate that matrix dynamics and cell metabolism/energy balance are processes that are affected by the electromagnetic field application.

In a follow-up study, using fibroblasts as in the study by Schwenzer et al. (2007), but exposing them to electric fields (EFs), Jennings et al. (2008) tried to elucidate the role of EFs during the course of normal wound healing. Fibroblasts at the wound edge are exposed to electric fields (EFs) ranging from 40 to 200 mV/mm and so various forms of EFs can influence fibroblast migration, proliferation, and protein synthesis and may contribute to fibroblast activation during wound repair. These authors compared gene expression in normal adult dermal fibroblasts exposed to a 100 mV/mm EF for 1 h to non-stimulated controls. Significantly increased expression of 162 transcripts and decreased expression of 302 transcripts was detected using microarrays, with 126 transcripts above the level of 1.4-fold increase or decrease compared to the controls. Only 11 genes were significantly increased or decreased above the level of 2-fold, compared to controls. Many of these significantly regulated genes were associated with wound repair through the processes of matrix production, cellular signalling, and growth. Activity within specific cellular signalling pathways was noted, including TGF-b, G-proteins, and inhibition of apoptosis. In addition, RT-PCR analysis of the expression of KLF6, FN1, RGS2, and JMJD1C over continued stimulation and at different field strengths suggests that there are specific windows of field characteristics for maximum induction in the expression of these genes. EFs thus appeared to have an important role in controlling fibroblast activity in the process of wound healing. The authors highlight that 2-fold changes have traditionally and somewhat arbitrarily been designated as meaningful changes in gene expression, although there is little quantitative information connecting these values to changes in biological function. Therefore, multiple microarray experiments at different time points and field conditions may have revealed induction of different sets of genes under different experimental conditions. Follow-up studies should include proteomic analysis of altered protein production resulting from altered gene expression, alternative splicing in protein translation, and gene silencing studies to further delineate the mechanisms and locations of interaction between EFs and transcriptional regulators.

Kimura et al. (2008) using magnetic resonance imaging with high intensity static magnetic fields (SMFs) demonstrated in the nematode *Caenorhabditis elegans* that genes involved in motor activity, actin binding, cell adhesion, and cuticles were transiently and specifically induced following exposure to 3 or 5 T SMF in this metazoon experimental model . In addition, transient induction of hsp12 family genes was observed after SMF exposure. The small-heat shock protein gene hsp16 was also induced but to a much lesser extent, and the LacZ-stained population of hsp-16.1::lacZ transgenic worms did not significantly increase after exposure to SMFs with or without a second stressor, mild heat shock. Several genes encoding apoptotic cell-death activators and secreted surface proteins were upregulated after IR, but were not induced by SMFs. Real-time quantitative RT-PCR analyses for 12 of these genes confirmed these expression differences between worms exposed to SMFs and IR. In contrast to IR, exposure to high SMFs did not induce DNA double-strand breaks or germline cell apoptosis during meiosis. These results suggest that the response of *C.*

elegans to high SMFs is unique and capable of adjustment during long exposure, and that this treatment may be less hazardous than other therapeutic tools.

On 2010, Chung et al. conducted a study to investigate the possible effect of 60 Hz circularly polarized magnetic fields (MFs) as promoters of genetically initiated lymphoma in AKR mice. One hundred sixty female animals were divided into four different groups. They were exposed to four different intensities of circularly polarized MFs. Animals received exposure to 60 Hz circularly polarized MF at field strengths (rms-value) of 0 microT (sham control, T1, Group I), 5 microT (T2, Group II), 83.3 microT (T3, Group III), or 500 microT (T4, Group IV), for 21 h/day from the age of 4-6 weeks to the age of 44-46 weeks. There were no exposure-related changes in mean survival time, clinical signs, body weights, hematological values, micronucleus assay, gene expression arrays, analysis of apoptosis, and necropsy findings. Examination at the histopathological level, showed lymphoma in all the groups. The tumor incidence was 31/40(78%), 30/40(75%), 32/40(80%), and 31/40(78%) in sham control, 5, 83.3, and 500 microT groups, respectively. However, there were no differences in the tumor incidence between the sham control (T1) and circularly polarized MF exposure groups (T2-T4). In conclusion, there was no evidence that exposure to 60 Hz circularly polarized MF strengths up to 500 microT promoted lymphoma in AKR mice.

In a very recent attempt to support a causative relationship between environmental exposure to extremely low-frequency electromagnetic fields (EMFs) at power line frequencies and the associated increase in risk of childhood leukemia, Kirschenlohr et al. (2012) tried to determine if gene expression changes occur in white blood cells of volunteers exposed to an ELF-EMF. Each of 17 pairs of male volunteers age 20-30 was subjected either to a 50 Hz EMF exposure of 62.0 ± 7.1 μT (approximately 600 mG) for 2 h or to a sham exposure (0.21 ± 0.05 μT) at the same time (11:00 a.m. to 13:00 p.m.). The alternative regime for each volunteer was repeated on the following day and the two-day sequence was repeated 6 days later, with the exception that a null exposure (0.085 ± 0.01 μT) replaced the sham exposure. Five blood samples (10 ml) were collected at 2 h intervals from 9:00 to 17:00 with five additional samples during the exposure and sham or null exposure periods on each study day. RNA samples were pooled for the same time on each study day for the group of 17 volunteers that were subjected to the ELF-EMF exposure/sham or null exposure sequence and were analyzed on Illumina microarrays. Time courses for 16 mammalian genes previously reported to be responsive to ELF-EMF exposure, including immediate early genes, stress response, cell proliferation and apoptotic genes were examined in detail. No genes or gene sets showed consistent response profiles to repeated ELF-EMF exposures. A stress response was detected as a transient increase in plasma cortisol at the onset of either exposure or sham exposure on the first study day. The cortisol response diminished progressively on subsequent exposures or sham exposures, and was attributable to mild stress associated with the experimental protocol.

Commenting the above data, we note that the overall experimental design seems to lack real life conditions since a) the suspicion refers to childhood leukaemia and not to adults, b) exposure is not supposed to be just 2 hours a day but day long for children living in the vicinity of power lines, c) continuous daily exposure for years is the rationale behind the possibility of ELFs causing or increasing leukaemia.

B. Proteomics

Proteins are the key molecules that participate and regulate nearly all cellular functions. The number of each protein species in a given cell changes over time according to the metabolic and signalling demand and is subject to differential gene expression. Proteomics, is the science that explores by high throughput techniques the so called “protein expression profile” of proteins.

The reports on ELF and proteomics are practically absent in the last 5 years leaving only the old study by Seyyedi et al. (2007) in human fibroblast (using 3 Hz, sinusoidal continuous ELF electromagnetic fields, 3 h duration and 4 mT magnetic field intensity) and one more in 2011 by Sulpizio et al. The first study showed that some protein expressions were affected by radiation after comparing the 2-DE separated proteins from the exposed and sham (control) cells. The two proteins that their expression was reduced about 50% were determined as alpha 1 antitrypsin (A1AT) and Transthyretin (TTR) and has been concluded that application of ELF-EMF in therapeutic aspects may be accompanied by their side effects.

Along the “leukaemia ELF rationale” and in addition a possible ELF link with cancer, cardiovascular, and neurological disorders, Sulpizio et al. (2011) exposed human SH-SY5Y neuroblastoma cells to a 50 Hz, 1 mT (10 Gauss) sinusoidal ELF-MF at three duration schemes, 5 days (T5), 10 days (T10), and 15 days (T15). The effects of ELF-MF on proteome expression and biological behavior were investigated. Through comparative analysis between treated and control samples they identified nine new proteins after a 15-day treatment. They suggested that the proteins were involved in a cellular defence mechanism and/or in cellular organization and proliferation such as peroxiredoxin isoenzymes (2, 3, and 6), 3-mercaptopyruvate sulfurtransferase, actin cytoplasmatic 2, t-complex protein subunit beta, ropporin-1A, and profilin-2 and spindlin-1. These authors concluded that ELF-MFs exposure altered the proliferative status and other important cell biology-related parameters, such as cell growth pattern, and cytoskeletal organization and that ELF radiation could trigger a shift toward a more invasive phenotype.

III. RADIOFREQUENCY ELECTROMAGNETIC FIELDS (RF-EMFS)

A relatively small number of publications have dealt after 2007 with the effects of RF-EMF on the proteome and transcriptome of cells and even less number with the effects on animals.

A. Transcriptomics

Chauhan et al. (2007a) assessed non-thermal RF-field exposure effects on a variety of biological processes (including apoptosis, cell cycle progression, viability and cytokine production) in a series of human-derived cell lines (TK6, HL60 and Mono-Mac-6). Exponentially growing cells were exposed to intermittent (5 min on, 10 min off) 1.9 GHz pulse-modulated RF fields for 6 h at mean specific absorption rates (SARs) of 0, 1 and 10 W/kg. Concurrent negative (incubator) and positive (heat shock for 1 h at 43 degrees C) controls were included in each experiment. Immediately after the 6-h exposure period and 18 h after exposure, cell pellets were collected and analyzed for cell viability, the incidence of apoptosis, and alterations in cell cycle kinetics. The cell culture supernatants were assessed for the presence of a series of human inflammatory cytokines (TNFA, IL1B, IL6, IL8, IL10, IL12) using a cytometric bead array assay. No detectable changes in cell viability, cell cycle kinetics, incidence of apoptosis, or cytokine expression were observed in any of RF-field-exposed groups in any of the cell lines tested, relative to the sham controls. However, the positive (heat-shock) control samples displayed a significant decrease in cell viability, increase in apoptosis, and alteration in cell cycle kinetics (G(2)/M block). Overall, the researchers found no evidence that non-thermal RF-field exposure could elicit any detectable biological effect in three human-derived cell lines.

Chauhan et al. (2007b) have examined the effect of RF field exposure on the possible expression of late onset genes in U87MG cells after a 24 h RF exposure period. In addition, a human monocyte-derived cell-line (Mono-Mac-6, MM6) was exposed to intermittent (5 min ON, 10 min OFF) RF fields for 6 h and then gene expression was assessed immediately after exposure and at 18 h post exposure. Both cell lines were exposed to 1.9 GHz pulse-modulated RF fields for 6 or 24 h at specific absorption rates (SARs) of 0.1-10.0 W/kg (very high SAR value). In support of their previous results, they found no evidence that nonthermal RF field exposure could alter gene expression in either cultured U87MG or MM6 cells, relative to non irradiated control groups. However, exposure of both cell-lines to heat-shock conditions (43 degrees C for 1 h) caused an alteration in the expression of a number of well-characterized heat-shock proteins.

The same year, Zhao et al. (2007) investigated whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to a working GSM cell phone rated at a frequency of 1900 MHz. Primary cultures were exposed for 2h. Microarray analysis and real-time RT-PCR were applied and showed up-regulation of caspase-2, caspase-6 and Asc gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons, but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The effects were specific since up-regulation was not seen for other genes associated with apoptosis, such as caspase-9 in either neurons or astrocytes, or Bax in neurons. The results showed that even relatively short-term exposure to cell phone radiofrequency emissions can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

In an *in vitro* study focusing on the effects of low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system, Hirose et al. (2007) tested the hypothesis that modulated RF fields act to induce phosphorylation and overexpression of heat shock protein hsp27. The study evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Secondly, the study investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz can induce activation or gene expression of hsp27 and other heat shock proteins (hsps). Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80 and 800 mW/kg for 2-48 h, and CW radiation at 80 mW/kg for 24 h. Human IMR-90 fibroblasts from fetal lungs were exposed to W-CDMA at 80 and 800 mW/kg for 2 or 28 h, and CW at 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the expression levels of phosphorylated hsp27 at serine 82 (hsp27[pS82]) were observed between the test groups exposed to W-CDMA or CW signal and the sham-exposed negative controls, as evaluated immediately after the exposure periods by bead-based multiplex assays. Moreover, no noticeable differences in the gene expression of hsps were observed between the test groups and the negative controls by DNA Chip analysis.

Paparini et al. (2008) found no evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal for 1 h at a whole body SAR of 1.1 W/kg. Gene expression was studied in the whole brain, where the average SAR was 0.2 W/kg, by expression microarrays containing over 22,600 probe sets. Comparison of data from sham and exposed animals showed no significant difference in gene expression modulation. However, when less stringent constraints were adopted to analyze microarray results, 75 genes were found to be modulated following exposure. Forty-two probes showed fold changes ranging from 1.5 to 2.8, whereas 33 were down-regulated from 0.67- to 0.29-fold changes, but these differences in gene expression were not confirmed by real-time PCR. Under these specific limited conditions, no consistent indication of gene expression changes in whole mouse brain was found associated to GSM 1800 MHz exposure. *We could possibly explain the lack of gene expression changes in this, as well in other studies, by the very short exposure duration used of 1 h.*

Nittby et al. (2008) applied Microarray hybridizations on Affymetrix rat2302 chips of RNA extracts from cortex and hippocampus of GSM 1800 exposed rats for just 6 h within TEM cells. Using four exposed and four control animals they found that a large number of genes were altered at hippocampus and cortex. The vast majority were downregulated. Since the genes that were differentially expressed between the two groups were responsible to membrane integral and signal transduction, the authors concluded that the change of their expression might be the cause of their previous observations of blood-brain-barrier leakage and albumin transport through brain capillaries.

Huang et al. (2008a) monitored cellular and molecular changes in Jurkat human T lymphoma cells after irradiating with 1763 MHz RF radiation in order to test the effect on RF radiation in immune cells. Jurkat T-cells were exposed to RF radiation to assess the effects on cell proliferation, cell cycle progression, DNA damage and gene expression. Cells were exposed to 1763 MHz RF radiation at 10 W/kg specific absorption rate (SAR) and compared to sham exposed cells. RF exposure did not produce significant changes in cell numbers, cell cycle distributions, or levels of DNA damage. In genome-wide analysis of gene expressions, there were no genes changed more than 2-fold upon RF-radiation while ten genes changed from 1.3 to approximately 1.8-fold. Among these ten genes, two cytokine receptor genes such as chemokine (C-X-C motif) receptor 3 (CXCR3) and interleukin 1 receptor, type II (IL1R2) were down-regulated upon RF radiation. These results indicate that the alterations in cell proliferation, cell cycle progression, DNA integrity or global gene expression were not detected upon 1763 MHz RF radiation under 10 W/kg SAR for 24 h to Jurkat T cells.

In a follow-up study Huang et al. (2008b) chose HEI-OC1 immortalized mouse auditory hair cells to characterize the cellular response to 1763 MHz RF exposure, because auditory cells can be exposed to mobile phone frequencies. Cells were exposed to 1763 MHz RF at a 20 W/kg specific absorption rate (SAR) in a code division multiple access (CDMA) exposure chamber for 24 and 48 h to check for changes in cell cycle, DNA damage, stress response, and gene expression. Neither cell cycle changes nor DNA damage were detected in RF-exposed cells. The expression of heat shock proteins (HSP) and the phosphorylation of mitogen-activated protein kinases (MAPK) did not change, either. The researchers tried to identify any alteration in gene expression using microarrays. Using the Applied Biosystems 1700 full genome expression mouse microarray, they found that 29 genes (0.09% of total genes examined) were changed by more than 1.5-fold on RF exposure. From these results, they could not find any evidence of the induction of cellular responses, including cell cycle distribution, DNA damage, stress response and gene expression, after 1763 MHz RF exposure at an SAR of 20 W/kg (very high value) in HEI-OC1 auditory hair cells.

Concerning plant cell experiments Engelmann et al. (2008) searched for physiological processes of plant cells sensitive to RF fields. They reported significant changes (but not more than 2.5-fold) in transcription of 10 genes in cell suspension cultures of *Arabidopsis thaliana*, which were exposed for 24 h to an RF field protocol representing typical microwave exposition in an urban environment. The changes in transcription of these genes were compared with published microarray datasets and revealed a weak similarity of the microwave to light treatment experiments. Considering the large changes described in published experiments, it is questionable if the small alterations caused by a 24 h continuous microwave exposure would have any impact on the growth and reproduction of whole plants.

Using very low SAR values (0.9–3 mWkg) Dawe et al. (2009) applied microarray technology in the nematode *C. elegans.* They compared five Affymetrix gene arrays of pooled triplicate RNA populations from sham-exposed L4/adult worms against five gene arrays of pooled RNA from microwave-exposed worms (taken from the same source population in each run). No genes showed consistent expression changes across all five comparisons, and all expression changes appeared modest after normalisation (< or =40% up- or down-regulated). The number of statistically significant differences in gene expression (846) was less than the false-positive rate expected by chance (1131). The authors concluded that the pattern of gene expression in L4/adult *C. elegans* is substantially unaffected by low-intensity microwave radiation and that the minor changes observed in this study could well be false positives. As a positive control, they compared RNA samples from N2 worms subjected to a mild heat-shock treatment (30 °C) against controls at 26 °C (two gene arrays per condition). As expected, heat-shock genes were strongly up-regulated at 30 °C, particularly an hsp-70 family member (C12C8.1) and hsp-16.2. Under these heat-shock conditions, they confirmed that an hsp-16.2::GFP transgene was strongly up-regulated, whereas two non-heat-inducible transgenes (daf-16::GFP; cyp-34A9::GFP) showed little change in expression. Preliminary work in our lab has indicated that this model organism is highly resistant to EMF sources including mobile phone, DECT and Wi-Fi radiation exposures, for reasons that are under investigation (Margaritis et al., unpublished).

RF exposure up to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines under these conditions as reported by Sekijima et al. (2010). These authors investigated the mechanisms by which radiofrequency (RF) fields exert their activity, and the changes in both cell proliferation and the gene expression profile in the human cell lines, A172 (glioblastoma), H4 (neuroglioma), and IMR-90 (fibroblasts from normal fetal lung) following exposure to 2.1425 GHz continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) RF fields at three field levels. During the incubation phase, cells were exposed at specific absorption rates (SARs) of 80, 250, or 800 mW/kg with both CW and W-CDMA RF fields for up to 96 h. Heat shock treatment was used as the positive control. No significant differences in cell growth or viability were observed between any test group exposed to W-CDMA or CW radiation and the sham-exposed negative controls. Using the Affymetrix Human Genome Array, only a very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression in each experiment. According to the authors the results confirm that low-level exposure to 2.1425 GHz CW and W-CDMA RF fields for up to 96 h did not act as an acute cytotoxicant in either cell proliferation or the gene expression profile. These results suggest that RF exposure up to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines under these conditions.

In order to investigate whether exposure to high-frequency electromagnetic fields (EMF) could induce adverse health effects, Trivino et al. (2012) cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM) in the presence of 900 MHz MW-EMF generated by a transverse electromagnetic (TEM) cell at short and long exposure times and the effect of high-frequency EMF on gene expression has been evaluated. Significant changes in gene expression levels of genes involved in DNA repair, cell cycle arrest, apoptosis, chromosomal organization, and angiogenesis were observed.The authors have identified functional pathways influenced by 900 MHz MW-EMF exposure.

It is worth mentioning, although beyond the frequencies used in cellular communication, that changes were detected using millimeter-waves in 56 genes at 6 h exposure and 58 genes at 24 h exposure in rats as shown by Millenbaugh et al. (2008). The animals were subjected to 35 GHz millimeter waves at a power density of 75 mW/cm^2, to sham exposure and to 42 degrees Centigrade environmental heat. Skin samples were collected at 6 and 24 h after exposure for Affymetrix Gene Chip analysis. The skin was harvested from a separate group of rats at 3-6 h or 24-48 h after exposure for histopathology analysis. Microscopic findings observed in the dermis of rats exposed to 35 GHz millimeter waves included aggregation of neutrophils in vessels, degeneration of stromal cells, and breakdown of collagen. Changes were detected in 56 genes at 6 h and 58 genes at 24 h in the millimeter-wave-exposed rats. Genes associated with regulation of transcription, protein folding, oxidative stress, immune response, and tissue matrix turnover were affected at both times. At 24 h, more genes related to extracellular matrix structure and chemokine activity were altered. Up-regulation of Hspa1a, Timp1, S100a9, Ccl2 and Angptl4 at 24 h by 35 GHz millimeter-wave exposure was confirmed by real-time RT-PCR. These results obtained from histopathology, microarrays and RT-PCR indicated that prolonged exposure to 35 GHz millimeter waves causes thermally related stress and injury in skin while triggering repair processes involving inflammation and tissue matrix recovery.

B. Proteomics

In a series of publications by Leszczynski's research group, consistently using human endothelial cell lines EA.hy926 and EA.hy926v1, protein expression changes occurred after exposure to 900 MHz.

The potential proteome expression changes by RF on the same cell line EA.hy926 have been further investigated by the same group in a follow-up study (Nylund et al., 2009), where they reported that 1h exposure to GSM 1800 MHz mobile phone radiation (SAR 2.0 W/kg) can also alter this cell line's proteome expression. Sham samples were produced simultaneously in the same conditions but without the radiation exposure. Cells were harvested immediately after 1-hour exposure to the radiation, and proteins were extracted and separated using 2-dimensional electrophoresis (2DE). In total, 10 experimental replicates were generated from both exposed and sham samples. About 900 protein spots were detected in the 2DE-gels using PDQuest software and eight of them were found to be differentially expressed in exposed cells ($p<0.05$, t-test). Three out of these eight proteins were identified using Maldi-ToF mass spectrometry (MS). These proteins were: spermidine synthase (SRM), 78 kDa glucose-regulated protein (55 kDa fragment) (GRP78) and proteasome subunit alpha type 1 (PSA1). Due to the lack of the availability of commercial antibodies the researchers were able to further examine expression of only GRP78. Using SDSPAGE and western blot method they were not able to confirm the result obtained for GRP78 using 2DE. Additionally, no effects were reported this time for 1800GSM exposure on the expression of vimentin and Hsp27 - proteins that were affected by the 900 MHz GSM exposure in their earlier studies. The authors highlight that the observed discrepancy between the expression changes of GRP78 detected with 1DE and 2DE confirms the importance of validation of the results obtained with 2DE using other methods, e.g. western blot.

Using a higher definition technique, the 2D-DIGE, Leszczynski's group investigated whether GSM1800 radiation can alter the proteome of primary human umbilical vein endothelial cells and primary human brain microvascular endothelial cells (Nylund et al., 2010). The cells were exposed for 1 hour to 1800 MHz GSM mobile phone radiation at an average specific absorption rate of 2.0 W/kg. Following that, cells were harvested immediately and the protein expression patterns of the sham-exposed and radiation-exposed cells were examined using two dimensional difference gel electrophoresis based proteomics (2DE-DIGE). Numerous differences were observed between the proteomes of human umbilical vein endothelial cells and human brain microvascular endothelial cells (both sham-exposed). These differences are most likely representing physiological differences between endothelia in different vascular beds. However, the exposure of both types of primary endothelial cells to mobile phone radiation did not cause any statistically significant changes in protein expression. So, radiation did not provoke any proteome expression changes to these kinds of cells immediately at the end of the exposure and when the false discovery rate correction was applied to analysis. This observation agrees with earlier the earlier study of this group showing that the 1800 MHz GSM radiation exposure had only very limited effect on the proteome of human endothelial cell line EA.hy926, as compared with the effect of 900 MHz GSM radiation.

Another "omics" group exposing human lens epithelial cells detected heat-shock protein (HSP) 70 and heterogeneous nuclear ribonucleoprotein K (hnRNP K) to be upregulated following exposure to GSM 1800 MHz for 2 h (Li et al., 2007). In three separate experiments, HLECs were exposed and sham-exposed (six dishes each) to 1800-MHz GSM-like radiation for 2 h. The specific absorption rates were 1.0, 2.0, or 3.5 W/kg. Immediately after radiation, the proteome was extracted from the HLECs. Immobilized pH gradient two-dimensional polyacrylamide gel electrophoresis (2-DE;

silver staining) and PDQuest 2-DE analysis software were used to separate and analyze the proteome of exposed and sham-exposed HLECs. Four differentially expressed protein spots were selected and identified by using electrospray ionization tandem mass spectrometry (ESI-MS-MS). When the protein profiles of exposed cells were compared with those of sham-exposed cells, four proteins were detected as upregulated. After analysis by ESI-MS-MS and through a database search, heat-shock protein (HSP) 70 and heterogeneous nuclear ribonucleoprotein K (hnRNP K) were determined to be upregulated in the exposed cells.

Since the above *in vitro* effects cannot be easily translated into humans, in 2008, Leszczynski's group performed a pilot study on volunteers (Karinen et al., 2008) and showed that mobile phone radiation might alter protein expression in human skin cells. Small area of forearm's skin in 10 female volunteers was exposed to RF-EMF (specific absorption rate SAR = 1.3 W/kg) and punch biopsies were collected from exposed and non-exposed areas of skin. Proteins extracted from biopsies were separated using 2-DE and protein expression changes were analyzed using PDQuest software. Analysis has identified 8 proteins that were statistically significantly affected (Anova and Wilcoxon tests). Two of the proteins were present in all 10 volunteers. This suggests that protein expression in human skin might be affected by the exposure to RF-EMF. The number of affected proteins was similar to the number of affected proteins observed in this group's earlier *in vitro* studies. This is the first study showing that molecular level changes might take place in human volunteers in response to exposure to RF-EMF, although the overall conclusions were criticized by Leszczynski et al. (2012).

However, such a limited and non systematic number of publications using "omics" approaches does not allow for any conclusions to be drawn concerning the impact of mobile phone emitted radiation upon cell proteome, physiology and function (Nylund et al., 2009), as also pointed out by Vanderstraeten & Verschaeve (2008).

Kim et al. (2010) have monitored changes in protein expression profiles in RF-exposed MCF7 human breast cancer cells using two-dimensional gel electrophoresis. MCF7 cells were exposed to 849 MHz RF radiation for 1 h per day for three consecutive days at specific absorption rates (SARs) of either 2 W/Kg or 10 W/kg. During exposure, the temperature in the exposure chamber was kept in an isothermal condition. Twenty-four hours after the final RF exposure, the protein lysates from MCF cells were prepared and two-dimensional electrophoretic analyses were conducted. The protein expression profiles of the MCF cells were not significantly altered as the result of RF exposure. None of the protein spots on the two-dimensional electrophoretic gels showed reproducible changes in three independent experiments. To determine effect of RF radiation on protein expression profiles more clearly, three spots showing altered expression without reproducibility were identified using electrospray ionization tandem mass spectrometry analysis and their expressions were examined with RT-PCR and Western blot assays. There was no alteration in their mRNA and protein levels. The authors concluded that it seems unlikely that RF exposure modulates the protein expression profile.

Since oxidative stress is gaining more and more ground as being the initial mechanism of action of EMFs, the review by Gaestel M. (2010) describes the (up to 2010) developments in analysing the influence of RF-EMFs on biological systems by monitoring the cellular stress response as well as overall gene expression. Recent data on the initiation and modulation of the classical cellular stress response by RF-EMFs, comprising expression of heat shock proteins and stimulation of stress-activated protein kinases, are summarised and evaluated. Since isothermic RF-EMF exposure is assumed rather than proven there are clear limitations in using the stress response to describe non-thermal effects of RF-EMFs. In particular, according to the authors further experiments are needed to characterise better the threshold of the thermal heat shock response and the homogeneity of the cellular response in the whole sample for each biological system used. Before then, it is proposed that the absence of the classical stress response can define isothermal experimental conditions and qualifies other biological effects of RF-EMFs detected under these conditions to be of non-thermal origin. To minimise the probability that by making this assumption valuable insights into the nature of biological effects of RF-EMFs could be lost, proteotoxic non-thermal RF-EMF effects should also be monitored by measuring activities of labile intracellular enzymes and/or levels of their metabolites before the threshold for the heat shock response is reached. In addition, non-thermal induction of the stress response via promoter elements distinct from the heat shock element (HSE) should be analysed using HSE-mutated heat shock promoter reporter constructs. Screening for non-thermal RF-EMF effects in the absence of a classical stress response should be performed by transcriptomics and proteomics. It is postulated that due to their high-throughput characteristics, these methods inherently generate false positive results and require statistical evaluation based on quantitative expression analysis from a sufficient number of independent experiments with identical parameters. In future approaches, positive results must be confirmed by independent quantitative methods and should also be evaluated *in vivo* to prove possible non-thermal effects of RF-EMFs on living beings. If successful, this strategy should contribute to identification of new underlying molecular mechanisms of interaction between RF-EMFs and living beings distinct from absorption of thermal energy.

In the review by Leszczynski et al., (2012) the authors have analyzed all available data up through the end of 2010 and have raised a number of concerns regarding the handling of proteomics technology, such as the different proteome analysis methods used, the low number of replicates, the posttreatment sampling (one or very few time points), the large number of protein analyzed, the huge differences in the dynamic range of protein concentrations in cells or plasma, the variety of posttranslational modifications, the lack of validation of the results with a second method, as well as the various SAR/exposure conditions/duration/frequency dependencies in order to properly evaluate the EMF impact. The authors agree along with Gerner et al. (2010) that protein expression per se may be a reliable way to explain EMF effects. We might add that in terms of protein synthesis dynamics, the quantity of any protein species at a given time point (as detected by proteomics) should take into account the protein stability and turnover (as pointed out by Eden et al., 2011) as well as mRNA stability and maturation/translational-posttranslational control. In a hypothetical scenario that EMFs affect gene activation /deactivation (see Blank & Goodman, 2008), the end effect may not be seen by proteomics, since no net quantity change is taking place immediately but (possibly) a few hours following exposure and (also hypothetically) normal levels come back a few days or weeks later due homeostatic mechanisms.

Our own contribution to the field of RF-EMF induced protein expression changes was performed in mice exposed to mobile phone and wireless DECT base radiation under real-time exposure conditions and analyzing thereafter the proteome of three critical brain regions; hippocampus, cerebellum and frontal lobe (Fragopoulou et al. 2012). Three equally divided groups of Balb/c mice (6 animals/group) were used; the first group was exposed to a typical mobile phone, at a SAR level range of 0.17-0.37 W/kg for 3 h daily for 8 months, the second group was exposed to a wireless DECT base (Digital Enhanced Cordless Telecommunications Telephone) at a SAR level range of 0.012-0.028 W/kg for 8 h/day for 8 months and the third group comprised the sham-exposed animals. Comparative proteomics analysis revealed that long-term irradiation from both EMF sources significantly altered ($p < 0.05$) the expression of 143 proteins in total (as low as 0.003 fold downregulation up to 114 fold overexpression). Several neural function related proteins (i.e., Glial Fibrillary Acidic Protein (GFAP), Alpha-synuclein, Glia Maturation Factor beta (GMF), and apolipoprotein E (apoE)), heat shock proteins, and cytoskeletal proteins (i.e., Neurofilaments and tropomodulin) are included in this list as well as proteins of the brain metabolism (i.e., Aspartate aminotransferase, Glutamate dehydrogenase) to nearly all brain regions studied. Western blot analysis on selected proteins confirmed the proteomics data. The observed protein expression changes may be related to brain plasticity alterations, indicative of oxidative stress in the nervous system or involved in apoptosis and might potentially explain human health hazards reported so far, such as headaches, sleep disturbance, fatigue, memory deficits, and long-term induction of brain tumors under similar exposure conditions.

As mentioned earlier, beyond the mobile phone frequencies, 35 GHz radiation had effects on gene expression. Similarly, Sypniewska et al. (2010) using proteomics reported that this frequency can also alter the proteome of NR8383 rat macrophages. Two-dimensional polyacrylamide gel electrophoresis, image analysis, and Western blotting were used to analyze approximately 600 protein spots in the cell lysates for changes in protein abundance and levels of 3-nitrotyrosine, a marker of macrophage stimulation. Proteins of interest were identified using peptide mass fingerprinting. Compared to plasma from sham-exposed rats, plasma from environmental heat- or millimeter wave-exposed rats increased the expression of 11 proteins, and levels of 3-nitrotyrosine in seven proteins, in the NR8383 cells. These altered proteins are associated with inflammation, oxidative stress, and energy metabolism. Findings of this study indicate both environmental heat and 35 GHz millimeter wave exposure elicit the release of macrophage-activating mediators into the plasma of rats.

Interestingly, there is a wealth of information regarding proteome and/or transcriptomics studies following exposure to ionizing radiation. In the perspective of similar mechanisms of action between NIR and IR, it is worth mentioning just one study using very low dose ionizing radiation by Pluder et al., 2011. In this study low-dose radiation induced rapid and time-dependent changes in the cytoplasmic proteome of the human endothelial cell line EA.hy926 (used by Dariusz Leszczynski and his group in their EMF studies). The proteomes were investigated at 4 and 24 h after irradiation at two different dose rates (Co-60 gamma ray total dose 200 mGy; 20 mGy/min and 190 mGy/min) using 2D-DIGE technology. The researchers identified 15 significantly differentially expressed proteins, of which 10 were upregulated and 5 down-regulated, with more than ± 1.5-fold difference compared with unexposed cells. Pathways influenced by the low-dose exposures included the Ran and RhoA pathways, fatty acid metabolism and stress response which are reminiscent of EMF impact studies.

Concerning proteomics techniques, a recent review by Damm et al., (2012) re-evaluates the putative advantages of microwave-assisted tryptic digests compared to conventionally heated protocols performed at the same temperature. An initial investigation of enzyme stability in a temperature range of 37-80°C demonstrated that trypsin activity declines sharply at temperatures above 60°C, regardless if microwave dielectric heating or conventional heating is employed. Tryptic digests of three proteins of different size (bovine serum albumin, cytochrome c and β-casein) were thus performed at 37°C and 50°C using both microwave and conventional heating applying accurate internal fiber-optic probe reaction temperature measurements. The impact of the heating method on protein degradation and peptide fragment generation was analyzed by SDS-PAGE and MALDI-TOF-MS. Time-dependent tryptic digestion of the three proteins and subsequent analysis of the corresponding cleavage products by MALDI-TOF provided virtually identical results for both microwave and conventional heating. In addition, the impact of electromagnetic field strength on the tertiary structure of trypsin and BSA was evaluated by molecular mechanics calculations. These simulations revealed that the applied field in a typical laboratory microwave reactor is 3-4 orders of magnitude too low to induce conformational changes in proteins or enzymes.

IV. SUMMARY

The papers analyzed in this review have dealt with a very difficult research problem, which is EMF effects as measured by the highthroughput techniques of transcriptomics and proteomics. It is a very difficult task because the technical complexity of the approaches is added to the enormous variations of the exposure details (duration, frequency, pulses, repetition, intensity, peak values, e.t.c). In total there were 29 original articles from 2007. Eight (8) of them were in the ELF frequencies, where the three of them indicate an effect in gene expression, the other three indicate no effect in gene expression and two studies show an effect in protein expression. Regarding radiofrequency studies (RF-EMF) a total of 21 papers were published in this area since 2007. Thirteen (13) dealt with transcriptomics [eight (8) effect- five (5) no effect] and eight (8) in proteomics [six (6) show effect and two (2) show no effect]. So, in total, 66% of the studies reveal an effect of EMF on transcriptome and proteome expression (Table 1).

Table 1
EMF Transcriptomics and Proteomics studies 2007-2012

(E=effect, NE= no effect)

The classification of the studies to the category "Effect – No effect" is based on the general conclusions of each article, although different conditions are used in exposure setup, biological system, duration, approaches. It is also considered as an effect even if a single gene or protein is affected by exposure to EMF.

	Exposed biological model	**Exposure set-up**	**SAR or/and power density or intensity of magnetic field**	**Duration of exposure / Time of sampling**	**Method of analysis**	**Category "Effect-No effect"**	**Comments**	**Reference/ Journal**
ELF –EMF Transcriptomics	**Primary human mesenchymal stem cells from the bone marrow and chondrocytes (cell line C28I2)**	BTEMF (combination of electromagnetic field and light therapy) Coil system	35 µT	Stimulated 5 times at 12-h intervals for 8 min each	Affymetrix GeneChip System, HG-U133A /RT-PCR partially confirmed the data	**E**	A limited number of regulated gene products from both cell types, which control cell metabolism and cell matrix structure,	Walther et al. (2007) *EBM*

							was mainly affected. There was no increased expression though of cancer-related genes	
	Adult human dermal fibroblasts *(scope: wound healing)*	Direct current field	100 mV/mm EF	1 h	Microarrays /RT-PCR validated 4 genes	**E**	Significantly increased expression of 162 transcripts and decreased expression of 302 transcripts was detected (126 transcripts above the level of 1.4-fold, 11 above the level of 2-	Jennings et al. (2008) *Bioelectromagnetics*

						fold)	
Caenorhabditis elegans	Static magnetic field (SMF) Magnetic resonance imaging	3 and 5 T	4 and 24 h	Affymetrix whole-genome array /qRT-PCR confirmed changes	**E**	Genes involved in motor activity, actin binding, cell adhesion, and cuticles, hsp12, hsp16 were transiently and specifically induced following exposure. Several genes encoding apoptotic cell-death activators and secreted surface proteins were	Kimura et al. (2008) *Bioelectromagnetics*

						upregulated after IR, but were not induced by SMFs.	
Embryonic human lung fibroblasts (Hel 299)	MR scanner	3.0 Tesla	2 h	cDNA microarray containing 498 known genes	**NE**		Schwenzer et al. (2007) *Journal of Magnetic Resonance imaging*
AKR mice	60 Hz Circularly polarized MFs	0 microT (sham control, T1, Group I), 5 microT (T2, Group II), 83.3 microT (T3, Group III), or 500 microT (T4, Group IV)	21 h/day from the age of 4-6 weeks to the age of 44-46 weeks	Affymetrix GeneChip Mouse Gene 1.0 ST assay	**NE**		Chung et al. (2010) *Bioelectromagnetics*
White blood cells of volunteers	50 Hz Sinusoidal ELF-MF	62.0 ± 7.1 µT	2 h, repeated on the followi	Illumina microarrays	**NE**		Kirschenlohr et al. (2012) *Radiat Res*

Proteomics				ng day and the two-day sequence was repeated 6 days later, 5 time points				
	Human fibroblasts	3 Hz continuous ELF, sinusoidal	4 mT	3 h	2-DE	**E**	Alpha 1 antitrypsin (A1AT) and Transthyretin (TTR) reduced their expression	Seyyedi et al. (2007) *Pak J Biol Sci*
	Human SH-SY5Y neuroblastoma cells	50 Hz Sinusoidal ELF-MF	1 mT	5, 10, 15 days	2-DE /Western blot and immuno - histochemical confirmation	**E**	Nine new proteins involved in cellular defence mechanism and/or in cellular organization	Sulpizio et al. (2011) *J Cell Biochem*

							and proliferation	
RF-EMF Transcriptomics	**Primary cultured neurons and astrocytes**	GSM 1900 MHz Real-life exposure conditions	Not calculated	2 h	Microarray analysis /RT-PCR	**E**	Up-regulation of caspase-2, caspase-6 and Asc gene expression in neurons and astrocytes (and Bax upregulation in astrocytes)	Zhao et al. (2007) *Neurosci Lett*
	Rat cortex and hippocampus	GSM mobile test phone at 1800 MHz	Whole-body SAR- 13 mW/kg brain SAR- 30 mW/kg	6 h	Microarray hybridizations on Affymetrix rat2302 chips	**E**	Altered gene categories in both cortex and hippocampus: extracellular region, signal transducer activity, intrinsic to	Nittby et al. (2008) *Environmentalist*

						membrane, and integral to membrane	
Jurkat human T lymphoma cells	1763 MHz CDMA exposure chamber	10 W/kg	24 h	Applied Biosystems microarrays	**E**	Ten genes changed from 1.3 to approximately 1.8-fold	Huang et al. (2008a) *Int J Radiat Biol*
HEI-OC1 immortalized mouse auditory hair cells	1763 MHz CDMA exposure chamber	20 W/kg	24 h, 48 h	Applied Biosystems 1700 full genome expression mouse microarray	**E**	29 genes (0.09% of total genes examined) were changed by more than 1.5-fold on RF exposure	Huang et al. (2008b) *Int J Radiat Biol*
Arabidopsis thaliana	RF field protocol representing typical microwave exposition in an urban environme	2 and 0.75 W/kg	24 h	RNA-extraction, microarray hybridization,and quantitative RT-PCR	**E**	Significant changes_(but not more than 2.5-fold) in transcription of 10 genes	Engelmann et al. (2008) *Computational Biology and Chemistry*

	nt						
Rats (skin)	35 GHz mm-waves	75 mW/cm^2	6 h, 24 h	Affymetrix Gene Chip analysis	**E**	Expression changes in 56 genes at 6 h exposure and 58 genes at 24 h exposure	Millenbaugh et al. (2008) *Radiat Res*
Cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM)	900 MHz CW TEM cells	3.5 mW/Kg 3 V/m 1 mW in the cell culture dishes	2 h and 48 h	cDNA-microarray analysis /Western blot confirmation	**E**	DNA repair genes activated from 2 hrs, apoptotic genes overexpressed, cell cycle arrest genes activated. Surprisingly effects with	Trivino et al. (2012) *EBM*

						very low dose	
Human cell lines, A172 (glioblastoma), H4 (neuroglioma), and IMR-90 (fibroblasts from normal fetal lung)	W-CDMA CW 2.1425 GHz	80, 250, or 800 mW/kg	For up to 96 h	Affymetrix Human Genome Array	**E**	A very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression	Sekijima et al. (2010) *J. Radiat. Res*
Human-derived cell lines (TK6, HL60 and Mono-Mac-6)	1.9 GHz pulse-modulated RF fields	0, 1 and 10 W/kg	Intermittent (5 min ON, 10 min OFF) for 6 h	Cell cycle, apoptosis, viability, cytokines tested at 0 and 18h after exposure	**NE**		Chauhan et al. (2007a) *Rad. Research*

	U87MG cells **Mono-Mac-6, MM6**	1.9 GHz pulse-modulated RF fields	0.1-10.0 W/kg	24 h intermittent (5 min ON, 10 min OFF) for 6 h	Microarrays analysis 18 h after exposure	**NE**		Chauhan et al. (2007b) *Proteomics*
	Human glioblastoma A172 cells **Human IMR-90 fibroblasts**	W-CDMA CW 2.1425 GHz	80 and 800 mW/kg 80 mW/kg 80 and 800 mW/kg 80 mW/kg	2-48 h 24 h 2h, 28h 28h	DNA Chip analysis	**NE**		Hirose et al. (2007) *Bioelectromagnetics*
	Mouse brain	GSM 1800 MHz	Whole body SAR of 1.1 W/kg	1 h	Microarrays containing over	**NE**	75 genes were found to be modulated	Paparini et al. (2008) *Bioelectro-magnetics*

			brain SAR 0.2 W/kg		22,600 probe sets RT-PCR		but since they were not confirmed ⇨ no effect	
	C. elegans	1.0 GHz, 0.5W power input	0.9–3 mW/kg	1.5h 2.5h	Five Affymetrix gene arrays of pooled triplicate RNA populations from L4/adult worms from each group (sham and exposed)	**NE**	Minor changes in gene expression, probably false positives. Strange intensity window effect, no effect in high dose.	Dawe et al. (2009) *Bioelectromagnetics*
Proteomics	**Human endothelial cell line EA.hy926**	GSM 1800 MHz	2.0 W/kg	1h	2-DE /Western blot confirmed selected proteins	**E**		Nylund et al. (2009) *Journal of Proteomics and Bioinformatics*

Human lens epithelial cells	GSM-like 1800-MHz	1.0, 2.0, or 3.5 W/kg.	2 h	2-DE	**E**	hnRNP K and HSP70 upregulated	Li et al. (2007) *Jpn. J. Ophtalmol*
Human skin cells.	Mobile phone GSM 900MHz	1.3 W/kg	1 h	2D in skin punch biopsies	**E**	8 proteins were affected	Karinen et al. (2008) *BMC Genomics*
Plasma from exposed rats causes changes in protein expression and levels of 3-NT in a rat alveolar macrophage cell line.NR8383 macrophages	Generator 35 GHz	Peak incident power density of 75 mW/cm^2	46 min	*in vitro* bioassay and proteomic screening	**E**	Increased the expression of 11 proteins, and levels of 3-nitrotyrosine in seven proteins, in the NR8383 cells. These altered proteins are associated with inflammation, oxidative stress, and energy metabolism	Sypniewska et al. (2010) *Bioelectromagnetics*
Human Jurkat T-	Modulated GSM 1800	2 W/kg	Intermittent	Autoradiography of 2-	**E**	Rate of protein	Gerner et al. (2010)

cells **Primary human diploid fibroblasts** **Peripheral blood mononuclear cells**	MHz		exposure 8h (5min ON 10 min OFF)	DE gel		synthesis in proliferating cells is increased by long-term (8 h) RF-EME, while no effect was detectable in quiescent white blood cells treated in the same manner.	*Int. Arch. Occup. Environ. Health*
Balb/c mice (hippocampus, frontal lobe, cerebellum)	GSM 900 MHz Mobile phone, 1880 MHz Wireless DECT base	0.17-0.37 W/kg 0.012-0.028 W/kg	3 h/day x 8 months 8 h/day x 8 months	2-De /Western blot confirmed selected proteins	**E**	Real-life exposure conditions	Fragopoulou et al. (2012) *EBM*
Human primary umbilical vein endothelial cells and	1800 MHz GSM	2.0 W/kg	1 h	2-DE	**NE**		Nylund et al. (2010) *Proteome Sci*

	primary human brain Microvascular endothelial cells							
	Human breast cancer MCF-7 cells	849 MHz CDMA	2 and 10 W/kg	1 h/day x 3 days	2D, 24 h after exposure, Rt-PCR, Western blot	**NE**		Kim et al. (2010) *J Radiat Res*

V. CONCLUSIONS

It is clear that the effects of EMFs are very difficult to predict in the cells, and that SAR values do not provide any information about the molecular mechanisms likely to take place during exposure. Unlike drugs, EMFs are absorbed in a variety of different, diverse and non-linear ways depending on the "microenvironment" receiving the radiation, the orientation of the molecular targets and their shape, the metabolic state at the moment of exposure, the energy absorbance at the microscale of the cell and the modulation of the waves. On this basis, it is rather difficult to replicate experiments under different conditions and cell systems, which may explain the discrepancy of the results among research groups.

As far as changes in gene expression are concerned, they are observed within specific time duration with and without recovery time. As mentioned in some studies i.e., the same endothelial cell line responded to 1800 MHz intermittent exposure, but not to continuous exposure. Exposure time, exposure pattern and type of biological system (organism, tissue, cell) and experimental techniques may also play a key role in the end effect (Mevissen M., 2011).

In addition, we point out that all "averaging approaches" like proteomics and transcriptomics provide a mean value of changes in a specific protein/gene from all cell types of the tissue examined. The same is true for western blotting, RT-PCR and the entire battery of biochemical/molecular biological techniques. Of course, newly developed high sensitivity proteomics and transcriptomics might be able to analyse small quantities from individual cell types, since cell protein/gene expression changes would be the approach of choice in future experiments utilizing sophisticated state of the art microscopical techniques. Under these conditions, we will be able to understand why one cell type responds to EMF whereas another cell type is not responding, thus leading to a net "no effect" in case the second cell type is outnumbered.

Therefore the issue of examining by proteomics various time points during (or after) exposure is of utmost importance in order to unravel the mechanism(s) of EMF action. Approaches including 2D-autoradiography might be in addition very useful in this direction since the actual protein synthetic profile will be revealed (Gerner et al., 2010). As stated by these authors their findings of an association between metabolic activity and the observed cellular reaction to low intensity RF-EMF may reconcile conflicting results of previous studies. They further postulated that the observed increased protein synthesis reflects an increased rate of protein turnover stemming from protein folding problems caused by the interference of radiofrequency electromagnetic fields with hydrogen bonds. These observations of course do not directly imply a health risk.

Needless to mention that a combination of all available high throughput techniques in the same system under identical exposure conditions will provide better data, especially if different laboratories replicate the results.

Taking into account that many studies using normal exposure conditions have revealed protein and gene expression changes, health hazards are possible.

It is clear that the existing guidelines are inadequate as pointed out by other studies as well (Fragopoulou et al., 2010). The transcriptomics and proteomics data reviewed here report that 66% of the papers published after 2007 show an effect. This is a clear indication of expression changes of proteins and genes at intensity levels commonly used by the wireless devices. Prudent avoidance of excessive usage of these devices is thus recommended.

Concerning the question of which model system is more suitable for such experiments in order to translate the effects into human EMF hazards, we might agree with Leszczynski's point that human volunteer skin is more suitable, but the major target of interest regarding EMF impacts is the brain which consists of an enormous complexity of nerve cell interactions far away from constituents of skin. Therefore, we argue that the system of choice for omics approaches should be rats or mice (preferably the second due to the possibility of handling transgenic material) as evolutionary very close to humans without neglecting the important work that has been (or will be) done using other biological systems, especially cell cultures.

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SECTION 6

Evidence For Genotoxic Effects

(RFR AND ELF Genotoxicity)

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Appendix 6-A - Abstracts on Effects of Extremely Low Frequency (ELF) on DNA showing Effect (E) and No Significant Effect (NE)

I. Introduction

Toxicity to the genome can lead to a change in cellular functions, cancer, and cell death. A large number of studies have been carried out to investigate the effects of electromagnetic field (EMF) exposure on DNA and chromosomal structures. The single-cell gel electrophoresis (comet assay) has been widely used to determine DNA damages: single and double strand breaks and cross-links. Studies have also been carried out to investigate chromosomal conformation and micronucleus formation in cells after exposure to EMF.

II. Radiofrequency radiation (RFR) and DNA damage (*28 total studies – 14 reported effects (50%) and 14 reported no significant effect (50%)*)

II A. DNA studies that reported effects*:*

The following is a summary of the research data reported in the literature.

Aitken et al. [2005] exposed mice to 900-MHz RFR at a specific absorption rate (SAR) of 0.09 W/kg for 7 days at 12 h per day. DNA damage in caudal epididymal spermatozoa was assessed by quantitative PCR (QPCR) as well as alkaline and pulsed-field gel electrophoresis postexposure. Gel electrophoresis revealed no significant change in single- or double-DNA strand breakage in spermatozoa. However, QPCR revealed statistically significant damage to both the mitochondrial genome ($p < 0.05$) and the nuclear β-globin locus ($p < 0.01$).

Diem et al [2005] exposed human fibroblasts and rat granulosa cells to mobile phone signal (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; during 4, 16 and 24 h; intermittent 5 min on/10min off or continuous). RFR exposure induced DNA single- and double-strand breaks as measured by the comet assay. Effects occurred after 16 h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect in the than continuous exposure.

Gandhi and Anita [2005] reported increases in DNA strand breaks and micronucleation in lymphocytes obtained from cell phone users.

Garaj-Vrhovac et al [1990] reported changes in DNA synthesis and structure in Chinese hamster cells after various durations of exposure to 7.7 GHz field at 30 mW/cm^2.

Lai and Singh [1995; 1996; 1997a; 2005] and Lai et al. [1997] reported increases in single and double strand DNA breaks in brain cells of rats exposed for 2 hrs to 2450-MHz field at 0.6-1.2 W/kg.

Lixia et al. [2006] reported an increase in DNA damage in human lens epithelial cells at 0 and 30 min after 2 hrs of exposure to 1.8 GHz field at 3 W/kg.

Markova et al. [2005] reported that GSM signals affected chromatin conformation and gama-H2AX foci that colocalized in distinct foci with DNA double strand breaks in human lymphocytes.

Narasimhan and Huh [1991] reported changes in lambdaphage DNA suggesting single strand breaks and strand separation.

Nikolova et al. [2005] reported a low and transient increase in DNA double strand break in mouse embryonic stem cells after acute exposure to 1.7- GHz field.

Paulraj and Behari [2006] reported an increased in single strand breaks in brain cells of rats after 35 days of exposure to 2.45 and 16.5 GHz fields at 1 and 2.01 W/kg.

Phillips et al. [1998] found increase and decrease in DNA strand breaks in cells exposure to various forms of cell phone radiation.

Sun et al. [2006] reported an increase in DNA single strand breaks in human lens epithelial cells after 2 hrs of exposure to 1.8 GHz field at 3 and 4 W/kg. The DNA damages caused by 4 W/kg field were irreversible.

Zhang et al. [2002] reported that 2450-MHz field at 5 mW/cm^2 did not induce DNA and chromosome damage in human blood cells after 2 hrs of exposure, but could increase DNA damage effect induced by mitomycin-C.

Zhang et al. [2006] reported that 1800-MHz field at 3.0 W/kg induced DNA damage in Chinese hamster lung cells after 24 hrs of exposure.

II B. DNA studies that reported no significant effect*:*

Chang et al. [2005] using the Ames assay found no significant change in mutation frequency in bacteria exposed for 48 hrs at 4W/kg to an 835-MHz CDMA signal.

Hook et al. [2004] showed that 24-hr exposure of Molt-4 cells to CDMA, FDMA, iDEN or TDMA modulated RF radiation did not significantly alter the level of DNA damage.

Lagroye et al. [2004a] reported no significant change in DNA strand breaks in brain cells of rats exposed for 2 hrs to 2450-MHz field at 1.2 W/kg.

Lagroye et al. [2004b] found no significant increases in DNA-DNA and DNA-protein cross-link in C3H10T(1/2) cells after a 2-hr exposure to CW 2450 MHz field at 1.9 W/kg.

Li et al. [2001] reported no significant change in DNA strand breaks in murine C3H10T(1/2) fibroblasts after 2 hrs of exposure to 847.74 and 835.02 MHz fields at 3-5 W/kg.

Maes et al. [1993, 1996, 1997, 2000, 2001, 2006] published a series of papers on in vitro genotoxic effects of radiofrequency radiation and interaction with chemicals. Their mostly found no significant effect.

Malyapa et al. [1997a,b, 1998] reported no significant change in DNA strand-breaks in cells exposed to 2450-Hz and various forms of cell phone radiation. Both in vitro and in vivo experiments were carried out.

McNamee et al. [2002a,b, 2003] found no significant increase in DNA breaks and micronucleus formation in human leukocytes exposed for 2 hrs to 1.9 GHz field at SAR up to 10 W/kg.

Sakuma et al. [2006] exposed human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs to mobile communication radiation for 2 and 24 hrs. No significant change in DNA strand breaks were observed up to 800 mW/kg.

Stronati et al. [2006] showed that 24 hrs of exposure to 935-MHz GSM basic signal at 1 or 2 W/Kg did not cause DNA strand breaks in human blood cells.

Tice et al. [2002] measured DNA single strand breaks in human leukocytes using the comet assay after exposure to various forms of cell phone signals. Cells were exposed at 37±1°C, for 3 or 24 h at average specific absorption rates (SARs) of 1.0-10.0 W/kg. Exposure for either 3 or 24 h did not induce a significant increase in DNA damage in leukocytes.

Vershaeve et al. [2006] long-term exposure (2 hrs/day, 5 days/week for 2 years) of rats to 900 MHz GSM signal at 0.3 and 0.9 W/kg did not significantly affect levels of DNA strand breaks in cells.

Vijayalaximi et al [2000] reported no significant increase in single strand breaks in human lymphocytes after 2 hrs of exposure to 2450-MHz field at 2 W/kg.

Zeni et al. [2005] reported that a 2-hr exposure to 900-MHz GSM signal at 0.3 and 1 W/kg did not significantly affect levels of DNA strand breaks in human leukocytes.

III. Micronucleus studies (29 Total studies: 16 reported effects (55%) and 13 reported no significant effect (45%))

III A. Micronucleus studies that reported effects:

Balode [1996] obtained blood samples from female Latvian Brown cows from a farm close to and in front of the Skrunda Radar and from cows in a control area. Micronuclei in peripheral erythrocytes were significantly higher in the exposed cows.

Busljeta et al. [2004] exposed male rats to 2.45 GHz RFR fields for 2 hours daily, 7 days a week, at 5-10 mW/cm^2 for up to 30 days. Erythrocyte count, haemoglobin and haematocrit were increased in peripheral blood on irradiation days 8 and 15. Anuclear cells and erythropoietic precursor cells were significantly decreased in the bone marrow on day 15, but micronucleated cells were increased.

D'Ambrosio et al. [2002] exposed human peripheral blood to 1.748 GHz continuous wave (CW) or phase-modulated wave (GMSK) for 15 min at a maximum specific absorption rate of ~5 W/kg. No changes were found in cell proliferation kinetics after exposure to either CW or GMSK fields. Micronucleus frequency result was not affected by CW exposure but a statistically significant increase in micronucleus was found following GMSK exposure.

Ferreira et al. [2006] found that rat offspring exposed to radiation from a cellular phone during their embryogenesis showed a significant increase in micronucleus frequency.

Fucic et al. [1992] reported increase in frequencies of micronuclei in the lymphocytes of humans exposed to microwaves.

Gandhi and Singh [2005] analyzed short term peripheral lymphocyte cultures for chromosomal aberrations and the buccal mucosal cells for micronuclei. They reported an increase in the number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes.

Garaj-Vrhovac et al [1992] exposed human whole-blood samples to continuous-wave 7.7 GHz radiation at power density of 0.5, 10 and 30 mW/cm^2 for 10, 30 and 60 min. In all experimental conditions, the frequencies of all types of chromosomal aberrations (dicentric and ring chromosomes) and micronucleus were significantly higher than in the control samples.

Garaj-Vrhovac et al. [1999] investigated peripheral blood lymphocytes of 12 subjects occupationally exposed to microwave radiation. Results showed an increase in frequency of micronuclei as well as disturbances in the distribution of cells over the first, second and third mitotic division in exposed subjects compared to controls.

Haider et al. [1994] exposed plant cuttings bearing young flower buds for 30 h on both sides of a slewable curtain antenna (300/500 kW, 40-170 V/m) and 15 m (90 V/m) and 30 m (70 V/m) distant from a vertical cage antenna (100 kW) as well as at the neighbors living near the broadcasting station (200 m, 1-3 V/m). Laboratory controls were maintained for comparison. Higher micronucleus frequencies than in laboratory controls were found for all exposure sites in the immediate vicinity of the antennae,

Tice et al. [2002] measured micronucleus frequency in human leukocytes using the comet assay after exposure to various forms of cell phone signals. Cells were exposed at 37±1°C, for 3 or 24 h at average specific absorption rates (SARs) of 1.0-10.0 W/kg. Exposure for 3 h did not induce a significant increase in micronucleated lymphocytes. However, exposure to each of the signals for 24 h at an average SAR of 5.0 or 10.0 W/kg resulted in a significant and reproducible increase in the frequency of micronucleated lymphocytes. The magnitude of the response (approximately four fold) was independent of the technology, the presence or absence of voice modulation, and the frequency.

Trosic et al. [2001] investigated the effect of a 2450-MHz microwave irradiation on alveolar macrophage kinetics and formation of multinucleated giant cells after whole body irradiation of rats at 5-15 mW/cm^2. A group of experimental animals was divided in four subgroups that received 2, 8, 13 and 22 irradiation treatments of two hours each. The animals were killed on experimental days 1, 8, 16, and 30. Multinucleated cells were significantly increased in treated animals. The increase in number of nuclei per cell was time- and dose-dependent. Macrophages with two nucleoli were more common in animals treated twice or eight times. Polynucleation was frequently observed after 13 or 22 treatments.

Trosic et al. [2002] exposed adult male Wistar for 2 h a day, 7 days a week for up to 30 days to continuous 2450-MHz microwaves at a power density of 5-10mW/cm^2. Frequency of micronuclei in polychromatic erythrocytes showed a significant increase in the exposed animals after 2, 8 and 15 days of exposure compared to sham-exposed control.

Trosic et al. [2004] investigated micronucleus frequency in bone marrow red cells of rats exposed to a 2450-MHz continuous–wave microwaves for 2 h daily, 7 days a week, at a power density of 5-10 mW/cm^2 (whole body SAR 1.25 +/- 0.36 (SE) W/kg). The frequency of micronucleated polychromatic erythrocytes was significantly increased on experimental day 15.

Trosic et al. [2006] exposed rats 2 h/day, 7 days/week to 2450-MHz microwaves at a whole-body SAR of 1.25 +/- 0.36W/kg. Control animals were included in the study. Bone marrow micronucleus frequency was increased on experimental day 15, and polychromatic erythrocytes micronucleus frequency in the peripheral blood was increased on day 8.

Zotti-Martelli et al. [2000] exposed human peripheral blood lymphocytes in G(0) phase to electromagnetic fields at different frequencies (2.45 and 7.7 GHz) and power densities (10, 20 and 30 mW/cm^2) for 15, 30 or 60min. The results showed for both radiation frequencies an induction of micronuclei as compared to control cultures at a power density of 30mW/cm^2 and after an exposure of 30 and 60 min.

Zotti-Martelli et al. [2005] exposed whole blood samples from nine different healthy donors for 60, 120 and 180 min to continuous-wave 1800-MHz microwaves at power densities of 5, 10 and 20 mW/cm^2. A statistically significant increase of micronucleus in lymphocytes was observed dependent on exposure time and power density. A considerable decrease in spontaneous and induced MN frequencies was measured in a second experiment.

III B. Micronucleus studies that reported no significant effects:

Bisht et al. [2002] exposed C3H 10T½ cells to 847.74 MHz CDMA (3.2 or 4.8 W/kg) or 835.62 MHz FDMA (3.2 or 5.1 W/kg) RFR for 3, 8, 16 or 24 h. No exposure condition was found to result in a significant increase relative to sham-exposed cells either in the percentage of binucleated cells with micronuclei or in the number of micronuclei per 100 binucleated cells.

Juutilainen et al. [2007] found no significant change in micronucleus frequency in erythrocytes of mice after long-term exposure to various mobile phone frequencies.

Koyama et al. [2004] exposed Chinese hamster ovary (CHO)-K1 cells to 2450-MHz microwaves for 2 h at average specific absorption rates (SARs) of 5, 10, 20, 50, 100, and 200 W/kg. Micronucleus frequency in cells exposed at SARs of 100 and 200 W/kg were significantly higher when compared with sham-exposed controls. They speculated that the effect observed was a thermal effect.

Port et al. [2003] reported that exposure of HL-60 cells to EMFs 25 times higher than the ICNIRP reference levels for occupational exposure did not induce any significant changes in apoptosis, micronucleation, abnormal morphologies and gene expression.

Scarfi et al [2006] exposed human peripheral blood lymphocytes to 900 MHz GSM signal at specific absorption rates of 0, 1, 5 and 10 W/kg peak values. No significant change in micronucleus frequency was observed.

Vijayalaximi et al. [1997a] exposed human blood to continuous-wave 2450- MHz microwaves, either continuously for a period of 90 min or intermittently for a total exposure period of 90 min (30 min on and 30 min off, repeated three times). The mean power density at the position of the cells was 5.0 mW/cm^2 and mean specific absorption rate was 12.46 W/kg. There were no significant differences between RFR-exposed and sham-exposed lymphocytes with respect to; (a) mitotic indices; (b) incidence of cells showing chromosome damage; (c) exchange aberrations; (d) acentric fragments; (e) binucleate lymphocytes, and (f) micronuclei.

Vijayalaximi et al. [1997b] exposed C3H/HeJ mice for 20 h/day, 7 days/week, over 18 months to continuous-wave 2450 MHz microwaves at a whole-body average specific absorption rate of 1.0 W/kg. At the end of the 18 months, peripheral blood and bone marrow smears were examined for the extent of genotoxicity as indicated by the presence of micronuclei in polychromatic erythrocytes. The results indicate that the incidence of micronuclei/1,000 polychromatic erythrocytes was not significantly different between groups exposed to RF radiation and sham-exposed groups.

Vijayalaximi et al. [1999] exposed CF-1 male mice to ultra-wideband electromagnetic radiation (UWBR) for 15 min at an estimated whole-body average specific absorption rate of 37 mW/kg. Peripheral blood and bone marrow smears were examined to determine the extent of genotoxicity, as assessed by the presence of micronuclei (MN) in polychromatic erythrocytes (PCE). There was no evidence for excess genotoxicity in peripheral blood or bone marrow cells of mice exposed to UWBR.

Vijayalaximi et al. [2001a] reported that there was no evidence for the induction of micronuclei in peripheral blood and bone marrow cells of rats exposed for 24h to 2450-MHz continuous-wave microwaves at a whole body average SAR of 12 W/kg.

Vijayalaximi et al. [2001b] reported that there is no evidence for the induction of chromosomal aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 835.62 MHz RF radiation at SARs of 4.4 or 5.0 W/kg.

Vijayalaximi et al. [2001c] reported no evidence for induction of chromosome aberrations and micronuclei in human blood lymphocytes exposed in vitro for 24 h to 847.74 MHz RF radiation (CDMA) at SARs of 4.9 or 5.5 W/kg.

Vijayalaximi et al. [2003] exposed timed-pregnant Fischer 344 rats (from nineteenth day of gestation) and their nursing offspring (until weaning) to a far-field 1.6 GHz Iridium wireless communication signal for 2 h/day, 7 days/week at power density of 0.43 mW/cm^2 and whole-body average specific absorption rate of 0.036 to 0.077 W/kg (0.10 to 0.22 W/kg in the brain). This was followed by chronic, head-only exposures of male and female offspring to a near-field 1.6 GHz signal for 2 h/day, 5 days/week, over 2 years. Near-field exposures were conducted at an SAR of 0.16 or 1.6 W/kg in the brain. At the end of 2 years, all rats were necropsied. Bone marrow smears were examined for the extent of genotoxicity, assessed from the presence of micronuclei in polychromatic erythrocytes. There was no evidence for excess genotoxicity in rats that were chronically exposed to 1.6 GHz microwaves compared to sham-exposed and cage controls.

Zeni et al. [2003] investigated the induction of micronucleus in human peripheral blood lymphocytes after exposure to electromagnetic fields at various duration of exposure, specific absorption rate (SAR), and signal [continuous-wave (CW) or GSM (Global System of Mobile Communication)-modulated signal]. No statistically significant difference was detected in any case.

IV. Chromosome and genome effects (21 studies total: 13 reported effects (62%) and 8 reported no significant effect (38%))

IV A. Chromosome and genome studies that reported effects:

Belyaev et al. [I992] studied the effect of low intensity microwaves on the conformational state of the genome of X-irradiated E. coli cells by the method of viscosity anomalous time dependencies. A power density of 1 microW/cm^2 is sufficient to suppress radiation-induced repair of the genome conformational state.

Belyaev et al. [1996] studied the effect of millimeter waves on the genome conformational state of E. coli AB1157 by the method of anomalous viscosity time dependencies in the frequency range of 51.64-51.85 GHz. Results indicate an electron-conformational interactions.

Belyaev et al. [2005] investigated response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to microwaves from GSM mobile phone (915 MHz, specific absorption rate 37 mW/kg), and power frequency magnetic field (50 Hz, 15 microT peak value). Changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected.

Belyaev et al. [2006] investigated whether exposure of rat brain to microwaves of global system for mobile communication (GSM) induces DNA breaks, changes in chromatin conformation and in gene expression at a specific absorption rate (SAR) of 0.4 mW/g for 2 h. Data showed that GSM MWs at 915 MHz did not induce DNA double stranded breaks detectable by pulsed-field gel electrophoresis or changes in chromatin conformation, but affected expression of genes in rat brain cells.

Gadhia et al. [2003] reported a significant increase in dicentric chromosomes in blood cells among mobile users who were smoker–alcoholic as compared to nonsmoker–nonalcoholic; the same held true for controls of both types.

Garaj-Vrhovac et al. [1990] exposed V79 Chinese hamster cells to continuous-wave 7.7 GHz RFR at power density of 30 mW/cm^2 for 15, 30, and 60 min. Results suggest that the radiation causes changes in the synthesis as well as in the structure of DNA molecules.

Garaj-Vrhovac et al. [1991] exposed V79 Chinese hamster fibroblast cells to continuous wave 7.7 GHz radiation at power density of 0.5 mW/cm^2 for 15, 30 and 60 min. There was a significantly higher frequency of specific chromosome aberrations such as dicentric and ring chromosomes in irradiated cells.

Mashevich et al. [2003] found that human peripheral blood lymphocytes exposed to continuous 830-MHz electromagnetic fields (1.6-8.8 W/kg for 72 hr) showed a SAR-dependent chromosome aneuploidy, a major "somatic mutation" leading to genomic instability and thereby to cancer. The aneuploidy was accompanied by an abnormal mode of replication of the chromosome 17 region engaged in segregation (repetitive DNA arrays associated with the centromere), suggesting that epigenetic alterations are involved in the SAR dependent genetic toxicity. The effects were non-thermal.

Ono et al. (2004) exposed pregnant mice intermittently at a whole-body averaged specific absorption rate of 0.71 W/kg (10 seconds on, 50 seconds off which is 4.3 W/kg during the 10 seconds exposure) for 16 hours a day, from the embryonic age of 0 to 15 days. At 10 weeks of age, mutation frequencies at the lacZ gene in spleen, liver, brain, and testis were examined. Quality of mutation assessed by sequencing the nucleotides of mutant DNAs revealed no appreciable difference between exposed and non-exposed samples.

Sarimov et al. [2004] reported that exposure to microwaves of 895-915 MHz at 5.4 mW/kg resulted in statistically significant changes in condensation of chromatin in human lymphocytes. Effects are similar to stress response, differ at various frequencies, and vary among donors.

Sarkar et al. [1994] exposed mice to 2450-MHz microwaves at a power density of 1 mW/cm^2 for 2 h/day over a period of 120, 150 and 200 days. Rearrangement of DNA segments were observed in testis and brain of exposed animals.

Semin et al. [1995] exposed DNA samples at 18^{o}C at 10 different microwave frequencies (4- to 8 GHz, 25 ms pulses, 0.4 to 0.7 mW/cm^2 peak power, 1- to 6-Hz repetition rate, no heating). Irradiation at 3 or 4 Hz and 0.6 mW/cm^2 peak power clearly increased the accumulated damage to the DNA secondary structure ($P< .00001$). However, changing the pulse repetition rate to 1, 5, 6 Hz, as well as changing the peak power to 0.4 or 0.7 mW/cm^2 did not induce significant effect. Thus, the effect occurred only within narrow ‘windows’ of the peak intensities and modulation frequencies.

Sykes et al. [2001] exposed mice daily for 30 min to plane-wave fields of 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms for 1, 5 or 25 days. Three days after the last exposure, spleen sections were screened for DNA inversion events. There was no significant difference between the control and treated groups in the 1- and 5-day exposure groups, but there was a significant reduction in inversions below the spontaneous frequency in the 25-day exposure group. This observation suggests that exposure to RF radiation can lead to a perturbation in recombination frequency which may have implications for recombination repair of DNA.

IV. B. Chromosome and genome studies that reported no significant effects:

Antonopoulos et al. [1997] found no significant change in cell cycle progression and the frequencies of sister-chromatid exchanges in human lymphocytes exposed to electromagnetic fields of 380, 900 and 1800 MHz.

Ciaravino et al. [1991] reported that RFR did not affect changes in cell progression caused by adriamycin, and the RFR did not change the number of sister chromatid exchanges that were induced by the adriamycin.

Garson et al. [1991] analyzed lymphocytes from Telecom Australia radio-linemen who had all worked with RFR in the range 400 kHz-20 GHz with exposures at or below the Australian occupational limits. There was no significant increase in chromosomal damage in circulating lymphocytes.

Gos et al. [2000] exposed actively growing and resting cells of the yeast Saccharomyces cerevisiae to 900-MHz Global System for Mobile Communication (GSM) pulsed modulation format signals at specific absorption rates (SAR) of 0.13 and 1.3 W/kg. They reported no significant effect of the fields on forward mutation rates on the frequency of petite formation, on rates of intrachromosomal deletion formation, or on rates of intragenic recombination in the absence or presence of the genotoxic agent methyl methansulfonate.

Kerbacher et al (1990) reported that exposure to pulsed 2450-MHz microwaves for 2 h at an SAR of 33.8 W/kg did not significantly cause chromosome aberrations in CHO cells. The radiation also did not interact with Mitomycin C and Adriamycin.

Komatsubara et al. [2005] reported that exposure to 2.45-GHz microwaves for 2 h with up to 100 W/kg SAR CW and an average 100 W/kg PW (a maximum SAR of 900 W/kg) did not induce chromosomal aberrations in mouse m5S cells.

Meltz et al. [1990] reported no significant mutagenic effect of exposure to 2.45-GHz RFR (40 W/kg) alone and interaction with proflavin, a DNA-intercalating drug, in L5178Y mouse leukemic cells.

Roti-Roti et al. [2001] reported no significant effect of exposure to radiofrequency radiation in the cellular phone communication range (835.62 MHz frequency division multiple access, FDMA; 847.74 MHz code division multiple access, CDMA) on neoplastic transformation frequency using the in vitro C3H 10T(1/2) cell transformation assay system.

Takahashi et al. [2002] exposed mice to 1.5 GHz EMF in the head region at 2.0, 0.67, and 0 W/kg specific absorption rate for 90 min/day, 5 days/week, for 4 weeks. No mutagenic effect in mouse brain cells was detected.

V. Conclusions

From this literature survey, since only 50% of the studies reported effects, it is apparent that there is no consistent pattern that radiofrequency radiation exposure could induce genetic damages/changes in cells and organisms. However, one can conclude that under certain conditions of exposure, radiofrequency radiation is genotoxic. Data available are mainly applicable only to cell phone radiation exposure. Other than the study by Phillips et al [1998], there is no indication that RFR at levels that one can experience in the vicinity of base stations and RF-transmission towers could cause DNA damage.

During cell phone use, a relatively constant mass of tissue in the brain is exposed to the radiation at relatively high intensity (peak SAR of 4 - 8 W/kg). Several studies reported DNA damage at lower than 4 W/kg. This questions the wisdom of the IEEE Committee in using 4 W/kg as the threshold of effect for exposure-standard setting. Furthermore, since critical genetic mutations in one single cell are sufficient to lead to cancer and there are millions of cells in a gram of tissue, it is inconceivable that the base of SAR standard was changed from averaged over 1 gm of tissue to 10 gm. (The limit of localized tissue exposure has been changed from 1.6 W/kg averaged over 1 gm of tissue to 2 W/kg over 10 gm of tissue. Since distribution of radiofrequency energy is non-homogenous inside tissue, this change allows a higher peak level of exposure.) What actually needed is a better refinement of SAR calculation to identify ‘peak values’ of SAR inside the brain,

Aside from influences that are not directly related to experimentation [Huss et al., 2007], many factors could influence the outcome of an experiment in bioelectromagnetics research.

Any effect of EMF has to depend on the energy absorbed by a biological entity and on how the energy is delivered in space and time. Frequency, intensity, exposure duration, and the number of exposure episodes can affect the response, and these factors can interact with each other to produce different effects. In addition, in order to understand the biological consequence of EMF exposure, one must know whether the effect is cumulative, whether compensatory responses result, and when homeostasis will break down. The contributions of these physical factors are discussed in a talk presented in

Vienna, Austria in 1998. The paper is posted in many websites (e.g., http://www.wave-guide.org/library/lai.html).

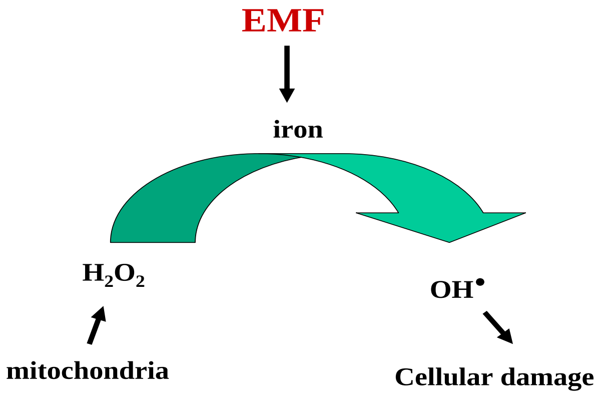
Thus, differences in outcomes of the research on genotoxic effects of RFR could be explained by the many different exposure conditions used in the studies. An example is the study of Phillips et al. [1998] showing that different cell phone signals could cause different effects on DNA (i.e., an increase in strand breaks with exposure to one type of signal and a decrease with another). This is further complicated by the fact that some of the studies listed above used very poor exposure procedures with very limited documentation of exposure parameters, e.g., using a cell phone to expose cells and even animals. Data from these experiments are questionable.

Another source of influence on an experimental outcome is the cell or organism studied. Many different biological systems were used in the genotoxicity studies. Different cell types [Hoyto et al., 2007] and organisms [Anderson et al., 2000; DiCarlo and Litovitz, 1999] may respond differently to EMF.

A few words have to be said on the ‘comet assay’, since it was used in most of the EMF studies to determine DNA damage. Different versions of the assay have been developed. These versions have different detection sensitivities and can be used to measure different aspects of DNA strand breaks. A comparison of data from experiments using different versions of the assay may be misleading. Another concern is that most of the ‘comet assay’ studies were carried out by experimenters who had no prior experience on the assay. My experience with the ‘comet assay’ is that it is a very sensitive assay and requires great care in performing. Thus, different detection sensitivities could result from different experimenters, even following the same procedures. One way to solve this experimental variation problem is for each researcher or laboratory to report their sensitivity of the ‘comet assay’, e.g., threshold of detecting strand breaks in human lymphocytes exposed to x-rays. This information is generally not available from the EMF-genotoxicity studies. However, in one incidence, an incredibly high sensitivity was even reported [Malyapa et al., 1998], suggesting the inexperience of the researchers on the assay.

A drawback in the interpretation and understanding of experimental data from bioelectromagnetic research is that there is no general acceptable mechanism on how EMF affects biological systems. The mechanism by which RFR causes genetic effect is unknown. Since the energy level is not sufficient to cause direct breakage of chemical bonds within molecules, the effects are probably indirect and secondary to other induced-chemical changes in the cell.

One possibility is via free radical formation inside cells. Free radicals kill cells by damaging macromolecules, such as DNA, protein and membrane. Several reports have indicated that electromagnetic fields (EMF) enhance free radical activity in cells [e.g., Lai and Singh, 1997a, b; 2004; Oral et al., 2006; Simko, 2007], particularly via the Fenton reaction [Lai and Singh, 2004]. The Fenton reaction is a catalytic process of iron to convert hydrogen peroxides, a product of oxidative respiration in the mitochondria, into hydroxyl free radical, which is a very potent and toxic free radical.



THE FENTON REACTION

What is interesting that extremely-low frequency EMF has also been shown to cause DNA damage (see the list of papers on ELF EMF and DNA at the end of this chapter). Free radicals have also been implicated in this effect of ELF EMF. This further supports the view that EMF affects DNA via an indirect secondary process, since the energy content of ELF EMF is much lower than that of RFR.

Effects via the Fenton reaction predict how a cell would respond to EMF:

1. Cells that are metabolic active would be more susceptible to the effect because more hydrogen peroxide is generated by the mitochondria to fuel the reaction.

2. Cells that have high level of intracellular free iron would be more vulnerable. Cancer cells and cells undergoing abnormal proliferation have high concentration of free iron because they uptake more iron and have less efficient iron storage regulation. Thus, these cells could be selectively damaged by EMF, and EMF could potentially be used for the treatment of cancer and hyperplasia diseases. The effect could be further enhanced if one could shift anaerobic glycolysis of cancer cells to oxidative glycolysis. There is quite a large database of information on the effects of EMF (mostly in the ELF range) on cancer cells and tumors. The data tend to indicate that EMF could retard tumor growth and kill cancer cells.

3. Since the brain is exposed to rather high levels of EMF during cell phone use, the consequences of EMF-induced genetic damage in brain cells are of particular importance. Brain cells have high level of iron. Special molecular pumps are present on nerve cell nucleus membrane to pump iron into the nucleus. Iron atoms have been found to intercalate within DNA molecules. In addition, nerve cells have a low capability for DNA repair and DNA breaks could accumulate. Another concern is the presence of superparamagnetic iron-particles (magnetites) in body tissues,

particularly in the brain. These particles could enhance free radical activity in cells and cellular-damaging effects of EMF. These factors make nerve cells more vulnerable to EMF. Thus, the effect of EMF on DNA could conceivably be more significant on nerve cells than on other cell types of the body. Since nerve cells do not divide and are not likely to become cancerous, more likely consequences of DNA damage in nerve cells are changes in functions and cell death, which could either lead to or accelerate the development of neurodegenerative diseases. Double strand breaks, if not properly repaired, are known to lead to cell death. Cumulative DNA damage in nerve cells of the brain has been associated with neurodegenerative diseases, such as Alzheimer's, Huntington's, and Parkinson's diseases. However, another type of brain cells, the glial cells, can become cancerous, resulting from DNA damage. The question is whether the damaged cells would develop into tumors before they are killed by EMF due to over accumulation of genetic damages. The outcome depends on the interplay of these different physical and biological factors: an increase, decrease, or no significant change in cancer risk could result.

4. On the other hand, cells with high antioxidant potentials would be less susceptible to EMF. These include the amount of antioxidants and anti-oxidative enzymes in the cells. Furthermore, the effect of free radicals could depend on the nutritional status of an individual, e.g., availability of dietary antioxidants, consumption of alcohol, and amount of food consumption. Various life conditions, such as psychological stress and strenuous physical exercise, have been shown to increase oxidative stress and enhance the effect of free radicals in the body. Thus, one can also speculate that some individuals may be more susceptible to the effects of EMF exposure.

More research has to be carried out to prove the involvement of the free radicals in the biological effects of EMF. However, the Fenton reaction obviously can only explain some the genetic effects observed. For example, RF- and ELF EMF-induced DNA damages have been reported in normal lymphocytes, which contain a very low concentration of intracellular free iron.

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VI. References for Radiofrequency Radiation Studies

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APPENDIX 6-A
Abstracts on Effects of Extremely Low Frequency (ELF) EMF on DNA

27 (E)- effect reported; 14 (NE)- no significant effect reported

Ahuja YR, **Vijayashree B**, **Saran R**, **Jayashri EL**, **Manoranjani JK**, **Bhargava SC**. **In vitro effects of low-level, low-frequency electromagnetic fields on DNA damage in human leucocytes by comet assay.** Indian J Biochem Biophys. 36(5):318-322, 1999. (E)

The sources for the effects of electromagnetic fields (EMFs) have been traced to time-varying as well as steady electric and magnetic fields, both at low and high to ultra high frequencies. Of these, the effects of low-frequency (50/60 HZ) magnetic fields, directly related to time-varying currents, are of particular interest as exposure to some fields may be commonly experienced. In the present study, investigations have been carried out at low-level (mT) and low-frequency (50 Hz) electromagnetic fields in healthy human volunteers. Their peripheral blood samples were exposed to 5 doses of electromagnetic fields (2,3,5,7 and 10mT at 50 Hz) and analysed by comet assay. The results were compared to those obtained from unexposed samples from the same subjects. 50 cells per treatment per individual were scored for comet-tail length which is an estimate of DNA damage. Data from observations among males were pooled for each flux density for analysis. At each flux density, with one exception, there was a significant increase in the DNA damage from the control value. When compared with a similar study on females carried out by us earlier, the DNA damage level was significantly higher in the females as compared to the males for each flux density.

Cantoni O, **Sestili P**, **Fiorani M**, **Dacha M**. **Effect of 50 Hz sinusoidal electric and/or magnetic fields on the rate of repair of DNA single strand breaks in cultured mammalian cells exposed to three different carcinogens: methylmethane sulphonate, chromate and 254 nm U.V. radiation.** Biochem Mol Biol Int. 38(3):527-533, 1996. (NE)

Treatment of cultured mammalian cells with three different carcinogens, namely methylmethane sulphonate (MMS), chromate and 254 U.V. radiation, produces DNA single strand breaks (SSB) in cultured mammalian cells. The rate of removal of these lesions is not affected by exposure to 50 Hz electric (0.2 - 20 kV/m), magnetic (0.0002-0.2 mT), or combined electric and magnetic fields. These results indicate that, under the experimental conditions utilized in this study, 50 Hz electric, magnetic and electromagnetic fields (over a wide range of intensities) do not affect the machinery involved in the repair of DNA SSBs generated by different carcinogens in three different cultured mammalian cell lines, making it unlikely that field exposure enhances the ability of these carcinogens to induce transformation via inhibition of DNA repair.

Chahal R, **Craig DQ**, **Pinney RJ**. **Investigation of potential genotoxic effects of low frequency electromagnetic fields on Escherichia coli.** J Pharm Pharmacol. 45(1):30-33, 1993. (NE)

Exposure of growing cells of Escherichia coli strain AB1157 to a frequency of 1 Hz with field strengths of 1 or 3 kV m-1 did not affect spontaneous or ultraviolet light (UV)-induced mutation frequencies to rifampicin resistance. Neither did growth in the presence of charge alter the sensitivities of strains AB1157, TK702 umuC or TK501 umuC uvrB to UV. Similarly, although the resistance of strains TK702 umuC and TK501 umuC uvrB to UV was increased by the presence of plasmid pKM101, which carries DNA repair genes, pregrowth of plasmid-containing strains in electric fields did not increase UV resistance. Finally, growth in a low frequency field in the presence of sub-inhibitory concentrations of mitomycin C did not affect mitomycin C-induced mutation frequencies. It is concluded that low frequency electromagnetic fields do not increase spontaneous mutation, induce DNA repair or increase the mutagenic effects of UV or mitomycin C.

Chow K, **Tung WL** **Magnetic field exposure enhances DNA repair through the induction of DnaK/J synthesis.** FEBS Lett. 478(1-2):133-136, 2000. (E)

In contrast to the common impression that exposure to a magnetic field of low frequency causes mutations to organisms, we have demonstrated that a magnetic field can actually enhance the efficiency of DNA repair. Using Escherichia coli strain XL-1 Blue as the host and plasmid pUC8 that had been mutagenized by hydroxylamine as the vector for assessment, we found that bacterial transformants that had been exposed to a magnetic field of 50 Hz gave lower percentages of white colonies as compared to transformants that had not been exposed to the magnetic field. This result was indicative that the efficiency of DNA repair had been improved. The improvement was found to be mediated by the induced overproduction of heat shock proteins DnaK/J (Hsp70/40).

Delimaris J, **Tsilimigaki S**, **Messini-Nicolaki N**, **Ziros E**, **Piperakis SM** Effects of pulsed electric fields on DNA of human lymphocytes. Cell Biol Toxicol. 22(6):409-415, 2006. (E)

The effects of pulsed electric fields of low frequency (50 Hz) on DNA of human lymphocytes were investigated. The influence of additional external factors, such as hydrogen peroxide (H_2O_2) and gamma-irradiation, as well as the repair efficiency in these lymphocytes, was also evaluated. The comet assay, a very sensitive and rapid method for detecting DNA damage at the single cells level was the method used. A significant amount of damage was observed after exposure to the electric fields, compared to the controls. After 2 h incubation at 37 degrees C, a proportion of damage was repaired. H_2O_2 and gamma-irradiation increased the damage to lymphocytes exposed to pulsed electric fields according to the dose used, while the amount of the repair was proportional to the damage.

Fairbairn DW, **O'Neill KL** **The effect of electromagnetic field exposure on the formation of DNA single strand breaks in human cells.** Cell Mol Biol (Noisy-le-grand). 40(4):561-567, 1994. (NE)

Electromagnetic fields (EMF) have been reported to be associated with human cancers in a number of epidemiological studies. Agents that are associated with cancer affect DNA in an adverse manner. This is a report of a DNA damage study in human cells exposed to EMFs. Single strand breaks in DNA are proposed to be necessary events in both mutagenesis and carcinogenesis. The single cell gel assay is a sensitive and accurate technique that was used in this study for single strand break detection. The EMF exposure system used here appeared to have no direct effect on DNA damage induction in a series of experiments. Moreover, EMF did not have a significant effect in potentiating DNA damage in cells treated with oxidative stresses.

Fiorani M, **Cantoni O**, **Sestili P**, **Conti R**, **Nicolini P**, **Vetrano F**, **Dacha M**. **Electric and/or magnetic field effects on DNA structure and function in cultured human cells.** Mutat Res. 282(1):25-29, 1992. (NE)

Exposure of cultured K562 cells to 50 Hz electric (0.2-20 kV/m), magnetic (0.002-2 G), or combined electric and magnetic fields for up to 24 h did not result in the production of detectable DNA lesions, as assayed by the filter elution technique. The rate of cell growth was also unaffected as well as the intracellular ATP and NAD+ levels. These results indicate that, under the experimental conditions utilized in this study, 50 Hz electric, magnetic and electromagnetic fields are not geno- and cyto-toxic in cultured mammalian cells.

Frazier ME, **Reese JA**, **Morris JE**, **Jostes RF**, **Miller DL** **Exposure of mammalian cells to 60-Hz magnetic or electric fields: analysis of DNA repair of induced, single-strand breaks.** Bioelectromagnetics. 11(3):229-234, 1990. (NE)

DNA damage was induced in isolated human peripheral lymphocytes by exposure at 5 Gy to 60Co radiation. Cells were permitted to repair the DNA damage while exposed to 60-Hz fields or while sham-exposed. Exposed cells were subjected to magnetic (B) or electric (E) fields, alone or in combination, throughout their allotted repair time. Repair was stopped at specific times, and the cells were immediately lysed and then analyzed for the presence of DNA single-strand breaks (SSB) by the alkaline-elution technique. Fifty to 75 percent of the induced SSB were repaired 20 min after exposure, and most of the remaining damage was repaired after 180 min. Cells were exposed to a 60-Hz ac B field of 1 mT; an E field of 1 or 20 V/m; or combined E and B fields of 0.2 V/m and 0.05 mT, 6 V/m and 0.6 mT, or 20 V/m and 1 mT. None of the exposures was observed to affect significantly the repair of DNA SSB.

Hong R, **Zhang Y**, **Liu Y**, **Weng EQ**. [Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 23(6):414-417, 2005. (E)

[Article in Chinese]

OBJECTIVE: To study the effects of 50 Hz electromagnetic fields (EMFs) on DNA of testicular cells and sperm chromatin structure in mice. METHODS: Mice were exposed to 50 Hz, 0.2 mT or 6.4 mT electromagnetic fields for 4 weeks. DNA strand breakage in testicular cells was detected by single-cell gel electrophoresis assay. Sperm chromatin structure was analyzed by sperm chromatin structure assay with flow cytometry. RESULTS: After 50 Hz, 0.2 mT or 6.4 mT EMFs exposure, the percentage of cells with DNA migration in total testicular cells increased from the control level of 25.64% to 37.83% and 39.38% respectively. The relative length of comet tail and the percentage of DNA in comet tail respectively increased from the control levels of 13.06% +/- 12.38% and 1.52% +/- 3.25% to 17.86% +/- 14.60% and 2.32% +/- 4.26% after 0.2 mT exposure and to 17.88% +/- 13.71% and 2.35% +/- 3.87% after 6.4 mT exposure ($P < 0.05$). Exposure to EMFs had not induced significant changes in S.D.alphaT and XalphaT, but COMPalphaT (cells outside the main population of alpha t), the percentage of sperms with abnormal chromatin structure, increased in the two exposed groups. CONCLUSION: 50 Hz EMFs may have the potential to induce DNA strand breakage in testicular cells and sperm chromatin condensation in mice.

Ivancsits S, **Pilger A**, **Diem E**, **Jahn O**, **Rudiger HW**.**Cell type-specific genotoxic effects of intermittent extremely low-frequency electromagnetic fields.** Mutat Res. 583(2):184-188, 2005. (E)

The issue of adverse health effects of extremely low-frequency electromagnetic fields (ELF-EMFs) is highly controversial. Contradictory results regarding the genotoxic potential of ELF-EMF have been reported in the literature. To test whether this controversy might reflect differences between the cellular targets examined we exposed cultured cells derived from different tissues to an intermittent ELF-EMF (50 Hz sinusoidal, 1 mT) for 1-24h. The alkaline and neutral comet assays were used to assess ELF-EMF-induced DNA strand breaks. We could identify three responder (human fibroblasts, human melanocytes, rat granulosa cells) and three non-responder cell types (human lymphocytes, human monocytes, human skeletal muscle cells), which points to the significance of the cell system used when investigating genotoxic effects of ELF-EMF.

Ivancsits S, **Diem E**, **Jahn O**, **Rudiger HW**. **Age-related effects on induction of DNA strand breaks by intermittent exposure to electromagnetic fields.** Mech Ageing Dev. 124(7):847-850, 2003. (E)

Several studies indicating a decline of DNA repair efficiency with age raise the question, if senescence per se leads to a higher susceptibility to DNA damage upon environmental exposures. Cultured fibroblasts of six healthy donors of different age exposed to intermittent ELF-EMF (50 Hz sinus, 1 mT) for 1-24 h exhibited different basal DNA strand break levels correlating with age. The cells revealed a maximum response at 15-19 h of exposure. This response was clearly more pronounced in cells from older donors, which could point to an age-related decrease of DNA repair efficiency of ELF-EMF induced DNA strand breaks.

Ivancsits S, **Diem E**, **Pilger A**, **Rudiger HW**, **Jahn O**. **Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts.** Mutat Res. 519(1-2):1-13, 2002. (E)

Results of epidemiological research show low association of electromagnetic field (EMF) with increased risk of cancerous diseases and missing dose-effect relations. An important component in assessing potential cancer risk is knowledge concerning any genotoxic effects of extremely-low-frequency-EMF (ELF-EMF).Human diploid fibroblasts were exposed to continuous or intermittent ELF-EMF (50Hz, sinusoidal, 24h, 1000microT). For evaluation of genotoxic effects in form of DNA single- (SSB) and double-strand breaks (DSB), the alkaline and the neutral comet assay were used.In contrast to continuous ELF-EMF exposure, the application of intermittent fields reproducibly resulted in a significant increase of DNA strand break levels, mainly DSBs, as compared to non-exposed controls. The conditions of intermittence showed an impact on the induction of DNA strand breaks, producing the highest levels at 5min field-on/10min field-off. We also found individual differences in response to ELF-EMF as well as an evident exposure-response relationship between magnetic flux density and DNA migration in the comet assay.Our data strongly indicate a genotoxic potential of intermittent EMF. This points to the need of further studies in vivo and consideration about environmental threshold values for ELF exposure.

Ivancsits S, **Diem E**, **Pilger A**, **Rudiger HW**, **Jahn O**. **Induction of DNA strand breaks by intermittent exposure to extremely-low-frequency electromagnetic fields in human diploid fibroblasts.** Mutat Res. 519(1-2):1-13, 2002. (E)

Results of epidemiological research show low association of electromagnetic field (EMF) with increased risk of cancerous diseases and missing dose-effect relations. An important component in assessing potential cancer risk is knowledge concerning any genotoxic effects of extremely-low-frequency-EMF (ELF-EMF).Human diploid fibroblasts were exposed to continuous or intermittent ELF-EMF (50Hz, sinusoidal, 24h, 1000microT). For evaluation of genotoxic effects in form of DNA single- (SSB) and double-strand breaks (DSB), the alkaline and the neutral comet assay were used.In contrast to continuous ELF-EMF exposure, the application of intermittent fields reproducibly resulted in a significant increase of DNA strand break levels, mainly DSBs, as compared to non-exposed controls. The conditions of intermittence showed an impact on the induction of DNA strand breaks, producing the highest levels at 5min field-on/10min field-off. We also found individual differences in response to ELF-EMF as well as an evident exposure-response relationship between magnetic flux density and DNA migration in the comet assay. Our data strongly indicate a genotoxic potential of intermittent EMF. This points to the need of further studies in vivo and consideration about environmental threshold values for ELF exposure.

Jajte J, **Zmyslony M**, **Palus J**, **Dziubaltowska E**, **Rajkowska E**. **Protective effect of melatonin against in vitro iron ions and 7 mT 50 Hz magnetic field-induced DNA damage in rat lymphocytes.** Mutat Res. 483(1-2):57-64, 2001. (E)

We have previously shown that simultaneous exposure of rat lymphocytes to iron ions and 50Hz magnetic field (MF) caused an increase in the number of cells with DNA strand breaks. Although the mechanism of MF-induced DNA damage is not known, we suppose that it involves free radicals. In the present study, to confirm our hypothesis, we have examined the effect of melatonin, an established free radicals scavenger, on DNA damage in rat peripheral blood lymphocytes exposed in vitro to iron ions and 50Hz MF. The alkaline comet assay was chosen for the assessment of DNA damage. During pre-incubation, part of the cell samples were supplemented with melatonin (0.5 or 1.0mM). The experiments were performed on the cell samples incubated for 3h in Helmholtz coils at 7mT 50Hz MF. During MF exposure, some samples were treated with ferrous chloride (FeCl2, 10microg/ml), while the rest served as controls. A significant increase in the number of cells with DNA damage was found only after simultaneous exposure of lymphocytes to FeCl2 and 7mT 50Hz MF, compared to the control samples or those incubated with FeCl2 alone. However, when the cells were treated with melatonin and then exposed to iron ions and 50Hz MF, the number of damaged cells was significantly reduced, and the effect depended on the concentration of melatonin. The reduction reached about 50% at 0.5mM and about 100% at 1.0mM. Our results indicate that melatonin provides protection against DNA damage in rat lymphocytes exposed in vitro to iron ions and 50Hz MF (7mT). Therefore, it can be suggested that free radicals may be involved in 50Hz magnetic field and iron ions-induced DNA damage in rat blood lymphocytes. The future experimental studies, in vitro and in vivo, should provide an answer to the question concerning the role of melatonin in the free radical processes in the power frequency magnetic field.

Kindzelskii AL, **Petty HR**. **Extremely low frequency pulsed DC electric fields promote neutrophil extension, metabolic resonance and DNA damage when phase-matched with metabolic oscillators.** Biochim Biophys Acta. 1495(1):90-111, 2000. (E)

Application of extremely low frequency pulsed DC electric fields that are frequency- and phase-matched with endogenous metabolic oscillations leads to greatly exaggerated neutrophil extension and metabolic resonance wherein oscillatory NAD(P)H amplitudes are increased. In the presence of a resonant field, migrating cell length grows from 10 to approximately 40 microm, as does the overall length of microfilament assemblies. In contrast, cells stop locomotion and become spherical when exposed to phase-mismatched fields. Although cellular effects were not found to be dependent on electrode type and buffer, they were sensitive to temporal constraints (phase and pulse length) and cell surface charge. We suggest an electromechanical coupling hypothesis wherein applied electric fields and cytoskeletal polymerization forces act together to overcome the surface/cortical tension of neutrophils, thus promoting net cytoskeletal assembly and heightened metabolic amplitudes. Metabolic resonance enhances reactive oxygen metabolic production by neutrophils. Furthermore, cellular DNA damage was observed after prolonged metabolic resonance using both single cell gel electrophoresis ('comet' assay) and 3'-OH DNA labeling using terminal deoxynucleotidyl transferase. These results provide insights into transmembrane signal processing and cell interactions with weak electric fields.

Lai H, **Singh NP**. **Acute exposure to a 60 Hz magnetic field increases DNA strand breaks in rat brain cells.** Bioelectromagnetics. 18(2):156-165, 1997. (E)

Acute (2 h) exposure of rats to a 60 Hz magnetic field (flux densities 0.1, 0.25, and 0.5 mT) caused a dose-dependent increase in DNA strand breaks in brain cells of the animals (assayed by a microgel electrophoresis method at 4 h postexposure). An increase in single-strand DNA breaks was observed after exposure to magnetic fields of 0.1, 0.25, and 0.5 mT, whereas an increase in double-strand DNA breaks was observed at 0.25 and 0.5 mT. Because DNA strand breaks may affect cellular functions, lead to carcinogenesis and cell death, and be related to onset of neurodegenerative diseases, our data may have important implications for the possible health effects of exposure to 60 Hz magnetic fields.

Lai H, **Singh NP**. **Magnetic-field-induced DNA strand breaks in brain cells of the rat.** Environ Health Perspect. 112(6):687-694, 2004. (E)

In previous research, we found that rats acutely (2 hr) exposed to a 60-Hz sinusoidal magnetic field at intensities of 0.1-0.5 millitesla (mT) showed increases in DNA single- and double-strand breaks in their brain cells. Further research showed that these effects could be blocked by pretreating the rats with the free radical scavengers melatonin and N-tert-butyl-alpha-phenylnitrone, suggesting the involvement of free radicals. In the present study, effects of magnetic field exposure on brain cell DNA in the rat were further investigated. Exposure to a 60-Hz magnetic field at 0.01 mT for 24 hr caused a significant increase in DNA single- and double-strand breaks. Prolonging the exposure to 48 hr caused a larger increase. This indicates that the effect is cumulative. In addition, treatment with Trolox (a vitamin E analog) or 7-nitroindazole (a nitric oxide synthase inhibitor) blocked magnetic-field-induced DNA strand breaks. These data further support a role of free radicals on the effects of magnetic fields. Treatment with the iron chelator deferiprone also blocked the effects of magnetic fields on brain cell DNA, suggesting the involvement of iron. Acute magnetic field exposure increased apoptosis and necrosis of brain cells in the rat. We hypothesize that exposure to a 60-Hz magnetic field initiates an iron-mediated process (e.g., the Fenton reaction) that increases free radical formation in brain cells, leading to DNA strand breaks and cell death. This hypothesis could have an important implication for the possible health effects associated with exposure to extremely low-frequency magnetic fields in the public and occupational environments.

Lai H, **Singh NP**. **Melatonin and N-tert-butyl-alpha-phenylnitrone block 60-Hz magnetic field-induced DNA single and double strand breaks in rat brain cells.** J Pineal Res. 22(3):152-162, 1997. (E)

In previous research, we have found an increase in DNA single- and double-strand breaks in brain cells of rats after acute exposure (two hours) to a sinusoidal 60-Hz magnetic field. The present experiment was carried out to investigate whether treatment with melatonin and the spin-trap compound N-tert-butyl-alpha-phenylnitrone (PBN) could block the effect of magnetic fields on brain cell DNA. Rats were injected with melatonin (1 mg/kg, sc) or PBN (100 mg/kg, ip) immediately before and after two hours of exposure to a 60-Hz magnetic field at an intensity of 0.5 mT. We found that both drug treatments blocked the magnetic field-induced DNA single- and double-strand breaks in brain cells, as assayed by a microgel electrophoresis method. Since melatonin and PBN are efficient free radical scavengers, these data suggest that free radicals may play a role in magnetic field-induced DNA damage.

Li SH, **Chow KC**. **Magnetic field exposure induces DNA degradation.** Biochem Biophys Res Commun. 280(5):1385-1388, 2001. (E)

In our earlier experiments, we discovered that magnetic field exposure could bring both stabilizing and destabilizing effects to the DNA of Escherichia coli, depending on our parameters of assessment, and both of these effects were associated with the induced synthesis of the heat shock proteins Hsp70/Hsp40 (DnaK/DnaJ). These contradicting results prompted us to explore in this study the effect of magnetic field exposure on the DNA stability in vivo when the heat shock response of the cell was suppressed. By using plasmid pUC18 in E. coli as the indicator, we found that without the protection of the heat shock response, magnetic field exposure indeed induced DNA degradation and this deleterious effect could be diminished by the presence of an antioxidant, Trolox C. In our in vitro test, we also showed that the magnetic field could potentiate the activity of oxidant radicals.

Lopucki M, **Schmerold I**, **Dadak A**, **Wiktor H**, **Niedermuller H**, **Kankofer M**. **Low dose magnetic fields do not cause oxidative DNA damage in human placental cotyledons in vitro.** Virchows Arch. 446(6):634-639, 2005. (NE)

The biological impact of low dose magnetic fields generated by electric appliances present in the human environment is still uncertain. In this study, human placentas served as a model tissue for the evaluation of the potential effect of oscillating low intensity magnetic fields on the concentration of 8-hydroxy-2'-deoxyguanosine (8-OH-dG) in cellular DNA. Cotyledons were dissected from placentas obtained immediately after physiological labours and exposed to magnetic fields (groups MF A, 2 mT, 50 Hz and MF B, 5 mT, 50 Hz) or sham exposed (group C) during an in vitro perfusion of 3 h. Cellular DNA was isolated, hydrolyzed and analyzed by HPLC. Native nucleosides were monitored at 254 nm and 8-OH-dG by electrochemical detection. Results were expressed as mumol 8-OH-dG/mol deoxyguanosine (dG). The concentrations of 8-OH-dG in group C, MF A and MF B were 28.45+/-15.27 micromol/mol dG, 62.80+/-31.91 mumol/mol dG, and 27.49+/-14.23 micromol/mol dG, respectively, demonstrating no significant difference between the groups. The results suggest that placental tissues possess a capacity to protect DNA against oxidative alterations by magnetic field of intensities previously shown to produce radical mediated DNA damage in rat brain cells in vivo and imbalances in electrolyte release of cotyledons under in vitro conditions.

Lourencini da Silva R, **Albano F**, **Lopes dos Santos LR**, **Tavares AD Jr**, **Felzenszwalb I**. **The effect of electromagnetic field exposure on the formation of DNA lesions.** Redox Rep. 5(5):299-301, 2000. (E)

In an attempt to determine whether electromagnetic field (EMF) exposure might lead to DNA damage, we exposed SnCl2-treated pBR322 plasmids to EMF and analysed the resulting conformational changes using agarose gel electrophoresis. An EMF-dependent potentiation of DNA scission (i.e. the appearance of relaxed plasmids) was observed. In confirmation of this, plasmids pre-exposed to EMF also were less capable of transforming Escherichia coli. The results indicate that EMF, in the presence of a transition metal, is capable of causing DNA damage. These observations support the idea that EMF, probably through secondary generation of reactive oxygen species, can be clastogenic and provide a possible explanation for the observed correlation between EMF exposure and the frequency of certain types of cancers in humans.

Luceri C, **De Filippo C**, **Giovannelli L**, **Blangiardo M**, **Cavalieri D**, **Aglietti F**, **Pampaloni M**, **Andreuccetti D**, **Pieri L**, **Bambi F**, **Biggeri A**, **Dolara P**. Extremely low-frequency electromagnetic fields do not affect DNA damage and gene expression profiles of yeast and human lymphocytes. Radiat Res. 164(3):277-285, 2005. (NE)

We studied the effects of extremely low-frequency (50 Hz) electromagnetic fields (EMFs) on peripheral human blood lymphocytes and DBY747 Saccharomyces cerevisiae. Graded exposure to 50 Hz magnetic flux density was obtained with a Helmholtz coil system set at 1, 10 or 100 microT for 18 h. The effects of EMFs on DNA damage were studied with the single-cell gel electrophoresis assay (comet assay) in lymphocytes. Gene expression profiles of EMF-exposed human and yeast cells were evaluated with DNA microarrays containing 13,971 and 6,212 oligonucleotides, respectively. After exposure to the EMF, we did not observe an increase in the amount of strand breaks or oxidated DNA bases relative to controls or a variation in gene expression profiles. The results suggest that extremely low-frequency EMFs do not induce DNA damage or affect gene expression in these two different eukaryotic cell systems.

McNamee JP, **Bellier PV**, **McLean JR**, **Marro L**, **Gajda GB**, **Thansandote A**. **DNA damage and apoptosis in the immature mouse cerebellum after acute exposure to a 1 mT, 60 Hz magnetic field.** Mutat Res. 513(1-2):121-133, 2002. (NE)

Several recent studies have reported that whole-body exposure of rodents to power frequency magnetic fields (MFs) can result in DNA single- and double-strand breaks in the brains of these animals. The current study was undertaken to investigate whether an acute 2h exposure of a 1 mT, 60 Hz MF could elicit DNA damage, and subsequently apoptosis, in the brains of immature (10-day-old) mice. DNA damage was quantitated at 0, 2, 4, and 24h after exposure using the alkaline comet assay. Apoptosis was quantitated in the external granule cell layer (EGCL) of the immature mouse cerebellum at 0 and 24h after exposure to MF by the TdT-mediated dUTP nick-end labeling (TUNEL) assay. Four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. While increased DNA damage was detected by tail ratio at 2h after MF exposure, no supporting evidence of increased DNA damage was detected by the other parameters. In addition, no similar differences were observed using these parameters at any of the other post-exposure times. No increase in apoptosis was observed in the EGCL of MF-exposed mice, when compared to sham mice. Taken together, these results do not support the hypothesis that acute MF exposure causes DNA damage in the cerebellums of immature mice.

McNamee JP, **Bellier PV**, **Chauhan V**, **Gajda GB**, **Lemay E**, **Thansandote A**. Evaluating DNA damage in rodent brain after acute 60 Hz magnetic-field exposure. Radiat Res. 164(6):791-797, 2005. (NE)

In recent years, numerous studies have reported a weak association between 60 Hz magnetic-field exposure and the incidence of certain cancers. To date, no mechanism to explain these findings has been identified. The objective of the current study was to investigate whether acute magnetic-field exposure could elicit DNA damage within brain cells from both whole brain and cerebellar homogenates from adult rats, adult mice and immature mice. Rodents were exposed to a 60 Hz magnetic field (0, 0.1, 1 or 2 mT) for 2 h. Then, at 0, 2 and 4 h after exposure, animals were killed humanely, their brains were rapidly removed and homogenized, and cells were cast into agarose gels for processing by the alkaline comet assay. Four parameters (tail ratio, tail moment, comet length and tail length) were used to assess DNA damage for each comet. For each species, a significant increase in DNA damage was detected by each of the four parameters in the positive control (2 Gy X rays) relative to the concurrent nonirradiated negative and sham controls. However, none of the four parameters detected a significant increase in DNA damage in brain cell homogenates from any magnetic-field exposure (0- 2 mT) at any time after exposure. The dose-response and time-course data from the multiple animal groups tested in this study provide no evidence of magnetic-field-induced DNA damage.

Miyakoshi J, **Yoshida M**, **Shibuya K**, **Hiraoka M**. **Exposure to strong magnetic fields at power frequency potentiates X-ray-induced DNA strand breaks.** J Radiat Res (Tokyo). 41(3):293-302, 2000. (E)

We examined the effect of an extremely low-frequency magnetic field (ELFMF) at 5, 50 and 400 mT on DNA strand breaks in human glioma MO54 cells. A DNA damage analysis was performed using the method of alkaline comet assay. The cells were exposed to X-rays alone (5 Gy), ELFMF alone, or X-rays followed by ELFMF at 4 degrees C or on ice. No significant difference in the tail moment was observed between control and ELFMF exposures up to 400 mT. X-ray irradiation increased DNA strand breaks. When cells were exposed to X-rays followed by ELFMF at 50 and 400 mT, the tail moment increased significantly compared with that for X-rays alone. When the exposure of cells was performed at 37 degrees C, no significant change was observed between X-rays alone and X-rays plus 400 mT. We previously observed that exposure to

400 mT ELFMF for 2 h increased X-ray-induced mutations (Miyakoshi et al, Mutat. Res., 349: 109-114, 1996). Additionally, an increase in the mutation by exposure to the ELFMF was observed in cells during DNA-synthesizing phase (Miyakoshi et al., Int. J. Radiat. Biol., 71: 75-79, 1997). From these results, it appears that exposure to the high density ELFMF at more than 50 mT may potentiate X-ray-induced DNA strand breaks.

Moretti M, **Villarini M**, **Simonucci S**, **Fatigoni C**, **Scassellati-Sforzolini G**, **Monarca S**, **Pasquini R**, **Angelucci M**, **Strappini M** **Effects of co-exposure to extremely low frequency (ELF) magnetic fields and benzene or benzene metabolites determined in vitro by the alkaline comet assay.** Toxicol Lett. 157(2):119-128, 2005. (E)

In the present study, we investigated in vitro the possible genotoxic and/or co-genotoxic activity of 50 Hz (power frequency) magnetic fields (MF) by using the alkaline single-cell microgel-electrophoresis (comet) assay. Sets of experiments were performed to evaluate the possible interaction between 50 Hz MF and the known leukemogen benzene. Three benzene hydroxylated metabolites were also evaluated: 1,2-benzenediol (1,2-BD, catechol), 1,4-benzenediol (1,4-BD, hydroquinone), and 1,2,4-benzenetriol (1,2,4-BT). MF (1 mT) were generated by a system consisting of a pair of parallel coils in a Helmholtz configuration. To evaluate the genotoxic potential of 50 Hz MF, Jurkat cell cultures were exposed to 1 mT MF or sham-exposed for 1h. To evaluate the co-genotoxic activity of MF, the xenobiotics (benzene, catechol, hydroquinone, and 1,2,4-benzenetriol) were added to Jurkat cells subcultures at the beginning of the exposure time. In cell cultures co-exposed to 1 mT (50 Hz) MF, benzene and catechol did not show any genotoxic activity. However, co-exposure of cell cultures to 1 mT MF and hydroquinone led to the appearance of a clear genotoxic effect. Moreover, co-exposure of cell cultures to 1 mT MF and 1,2,4-benzenetriol led to a marked increase in the genotoxicity of the ultimate metabolite of benzene. The possibility that 50 Hz (power frequency) MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

Nikolova T, **Czyz J**, **Rolletschek A**, **Blyszczuk P**, **Fuchs J**, **Jovtchev G**, **Schuderer J**, **Kuster N**, **Wobus AM**. **Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells.** ASEB J. 19(12):1686-1688, 2005. (E)

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

Reese JA, **Jostes RF**, **Frazier ME**. **Exposure of mammalian cells to 60-Hz magnetic or electric fields: analysis for DNA single-strand breaks.** Bioelectromagnetics. 9(3):237-247, 1998. (NE)

Chinese hamster ovary (CHO) cells were exposed for 1 h to 60-Hz magnetic fields (0.1 or 2 mT), electric fields (1 or 38 V/m), or to combined magnetic and electric fields (2 mT and 38 V/m, respectively). Following exposure, the cells were lysed, and the DNA was analyzed for the presence of single-strand breaks (SSB), using the alkaline elution technique. No significant differences in numbers of DNA SSB were detected between exposed and sham-exposed cells. A positive control exposed to X-irradiation sustained SSB with a dose-related frequency. Cells exposed to nitrogen mustard (a known cross-linking agent) and X-irradiation demonstrated that the assay could detect cross-linked DNA under our conditions of electric and magnetic field exposures.

Robison JG, **Pendleton AR**, **Monson KO**, **Murray BK**, **O'Neill KL**. **Decreased DNA repair rates and protection from heat induced apoptosis mediated by electromagnetic field exposure.** Bioelectromagnetics. 23(2):106-112, 2002. (E)

In this study, we demonstrate that electromagnetic field (EMF) exposure results in protection from heat induced apoptosis in human cancer cell lines in a time dependent manner. Apoptosis protection was determined by growing HL-60, HL-60R, and Raji cell lines in a 0.15 mT 60 Hz sinusoidal EMF for time periods between 4 and 24 h. After induction of apoptosis, cells were analyzed by the neutral comet assay to determine the percentage of apoptotic cells. To discover the duration of this protection, cells were grown in the EMF for 24 h and then removed for 24 to 48 h before heat shock and neutral comet assays were performed. Our results demonstrate that EMF exposure offers significant protection from apoptosis ($P<.0001$ for HL-60 and HL-60R, $P<.005$ for Raji) after 12 h of exposure and that protection can last up to 48 h after removal from the EMF. In this study we further demonstrate the effect of the EMF on DNA repair rates. DNA repair data were gathered by exposing the same cell lines to the EMF for 24 h before damaging the exposed cells and non-exposed cells with H2O2. Cells were allowed to repair for time periods between 0 and 15 min before analysis using the alkaline comet assay. Results showed that EMF exposure significantly decreased DNA repair rates in HL-60 and HL-60R cell lines ($P<.001$ and $P<.01$ respectively), but not in the Raji cell line. Importantly, our apoptosis results show that a minimal time exposure to an EMF is needed before observed effects. This may explain previous studies showing no change in apoptosis susceptibility and repair rates when treatments and EMF exposure were administered concurrently. More research is necessary, however, before data from this in vitro study can be applied to in vivo systems.

Scarfi MR, **Sannino A**, **Perrotta A**, **Sarti M**, **Mesirca P**, **Bersani F**. **Evaluation of genotoxic effects in human fibroblasts after intermittent exposure to 50 Hz electromagnetic fields: a confirmatory study.** Radiat Res. 164(3):270-276, 2005. (NE)

The aim of this investigation was to confirm the main results reported in recent studies on the induction of genotoxic effects in human fibroblasts exposed to 50 Hz intermittent (5 min field on/10 min field off) sinusoidal electromagnetic fields. For this purpose, the induction of DNA single-strand breaks was evaluated by applying the alkaline single-cell gel electrophoresis (SCGE)/comet assay. To extend the study and validate the results, in the same experimental conditions, the potential genotoxicity was also tested by exposing the cells to a 50 Hz powerline signal (50 Hz frequency plus its harmonics). The cytokinesis-block micronucleus assay was applied after 24 h intermittent exposure to both sinusoidal and powerline signals to obtain information on cell cycle kinetics. The experiments were carried out on human diploid fibroblasts (ES-1). For each experimental run, exposed and sham-exposed samples were set up; positive controls were also provided by treating cells with hydrogen peroxide or mitomycin C for the comet or micronucleus assay, respectively. No statistically significant difference was detected in exposed compared to sham-exposed samples in any of the experimental conditions tested ($P > 0.05$). In contrast, the positive controls showed a statistically significant increase in DNA damage in all cases, as expected. Accordingly, our findings do not confirm the results reported previously for either comet induction or an increase in micronucleus frequency.

Schmitz C, **Keller E**, **Freuding T**, **Silny J**, **Korr H**. **50-Hz magnetic field exposure influences DNA repair and mitochondrial DNA synthesis of distinct cell types in brain and kidney of adult mice.** Acta Neuropathol (Berl). 107(3):257-264, 2004. (E)

Despite several recent investigations, the impact of whole-body magnetic field exposure on cell-type-specific alterations due to DNA damage and DNA repair remains unclear. In this pilot study adult mice were exposed to 50-Hz magnetic field (mean value 1.5 mT) for 8 weeks or left unexposed. Five minutes after ending exposure, the mice received [(3)H]thymidine and were killed 2 h later. Autoradiographs were prepared from paraffin sections of brains and kidneys for measuring unscheduled DNA synthesis and mitochondrial DNA synthesis, or in situ nick translation with DNA polymerase-I and [(3)H]dTTP. A significant ($P<0.05$) increase in both unscheduled DNA synthesis and in situ nick translation was only found for epithelial cells of the choroid plexus. Thus, these two independent methods indicate that nuclear DNA damage is produced by long-lasting and strong magnetic field exposure. The fact that only plexus epithelial cells were affected might point to possible effects of magnetic fields on iron transport across the blood-cerebrospinal fluid barrier, but the mechanisms are currently not understood. Mitochondrial DNA synthesis was exclusively increased in renal epithelial cells of distal convoluted tubules and collecting ducts, i.e., cells with a very high content of mitochondria, possibly indicating increased metabolic activity of these cells.

Singh N, **Lai H**. **60 Hz magnetic field exposure induces DNA crosslinks in rat brain cells.** Mutat Res. 400(1-2):313-320, 1998. (E)

In previous research, we found an increase in DNA strand breaks in brain cells of rats acutely exposed to a 60 Hz magnetic field (for 2 h at an intensity of 0.5 mT). DNA strand breaks were measured with a microgel electrophoresis assay using the length of DNA migration as an index. In the present experiment, we found that most of the magnetic field-induced increase in DNA migration was observed only after proteinase-K treatment, suggesting that the field caused DNA-protein crosslinks. In addition, when brain cells from control rats were exposed to X-rays, an increase in DNA migration was observed, the extent of which was independent of proteinase-K treatment. However, the X-ray-induced increase in DNA migration was retarded in cells from animals exposed to magnetic fields even after proteinase-K treatment, suggesting that DNA-DNA crosslinks were also induced by the magnetic field. The effects of magnetic fields were also compared with those of a known DNA crosslink-inducing agent mitomycin C. The pattern of effects is similar between the two agents. These data suggest that both DNA-protein and DNA-DNA crosslinks are formed in brain cells of rats after acute exposure to a 60 Hz magnetic field.

Stronati L, **Testa A**, **Villani P**, **Marino C**, **Lovisolo GA**, **Conti D**, **Russo F**, **Fresegna AM**, **Cordelli E** **Absence of genotoxicity in human blood cells exposed to 50 Hz magnetic fields as assessed by comet assay, chromosome aberration, micronucleus, and sister chromatid exchange analyses.** Bioelectromagnetics. 25(1):41-48, 2004. (NE)

In the past, epidemiological studies indicated a possible correlation between the exposure to ELF fields and cancer. Public concern over possible hazards associated with exposure to extremely low frequency magnetic fields (ELFMFs) stimulated an increased scientific research effort. More recent research and laboratory studies, however, have not been able to definitively confirm the correlation suggested by epidemiological studies. The aim of this study was to evaluate the effects of 50 Hz magnetic fields in human blood cells exposed in vitro, using several methodological approaches for the detection of genotoxicity. Whole blood samples obtained from five donors were exposed for 2 h to 50 Hz, 1 mT uniform magnetic field generated by a Helmholtz coil system. Comet assay, sister chromatid exchanges (SCE), chromosome aberrations (CA), and micronucleus (MN) tests were used to assess DNA damage, one hallmark of malignant cell transformation. The effects of a combined exposure with X-rays were also evaluated. Results obtained do not show any significant difference between ELFMFs exposed and unexposed samples. Moreover, no synergistic effect with ionizing radiation has been observed. A slight but significant decrease of cell proliferation was evident in ELFMFs treated samples and samples subjected to the combined exposure.

Svedenstal BM, **Johanson KJ**, **Mild KH**. **DNA damage induced in brain cells of CBA mice exposed to magnetic fields.** In Vivo. 13(6):551-552, 1999. (E)

DNA migration, using single cell gel electrophoresis (comet assay), was studied on brain cells of CBA mice exposed continuously to 50 Hz, 0.5 mT magnetic fields (MF) for 2 hrs, 5 days or 14 days. No differences were observed in the groups MF-exposed for 2 hrs and 5 days compared with controls. However, in the group exposed to MF for 14 days, a significantly extended cell DNA migration was observed ($0.02 < p < 0.05$). These changes together with results from previous studies indicate that magnetic fields may have genotoxic effects in brain cells.

Testa A, **Cordelli E**, **Stronati L**, **Marino C**, **Lovisolo GA**, **Fresegna AM**, **Conti D**, **Villani P**. **Evaluation of genotoxic effect of low level 50 Hz magnetic fields on human blood cells using different cytogenetic assays.** Bioelectromagnetics. 25(8):613-619, 2004. (NE)

The question whether extremely low frequency magnetic fields (ELFMFs) may contribute to mutagenesis or carcinogenesis is of current interest. In order to evaluate the possible genotoxic effects of ELFMFs, human blood cells from four donors were exposed in vitro for 48 h to 50 Hz, 1 mT uniform magnetic field generated by a Helmholtz coil system. Comet assay (SCGE), sister chromatid exchanges (SCE), chromosome aberrations (CAs), and micronucleus (MN) test were used to assess the DNA damage. ELF pretreated cells were also irradiated with 1 Gy of X-ray to investigate the possible combined effect of ELFMFs and ionizing radiation. Furthermore, nuclear division index (NDI) and proliferation index (PRI) were evaluated. Results do not evidence any DNA damage induced by ELFMF exposure or any effect on cell proliferation. Data obtained from the combined exposure to ELFMFs and ionizing radiation do not suggest any synergistic or antagonistic effect.

Villarini M, **Moretti M**, **Scassellati-Sforzolini G**, **Boccioli B**, **Pasquini R**. Effects of co-exposure to extremely low frequency (50 Hz) magnetic fields and xenobiotics determined in vitro by the alkaline comet assay. Sci Total Environ. 361(1-3):208-219, 2006. (E)

In the present study, we used human peripheral blood leukocytes from 4 different donors, to investigate in vitro the possible genotoxic and/or co-genotoxic activity of extremely low frequency magnetic fields (ELF-MF) at 3 mT intensity. Two model mutagens were used to study the possible interaction between ELF-MF and xenobiotics: N-methyl-N'-nitro-N-nitrosoguanidine (MNNG) and 4-nitroquinoline N-oxide (4NQO). Primary DNA damage was evaluated by the alkaline single-cell microgel-electrophoresis ("comet") assay. Control cells (leukocytes not exposed to ELF-MF, nor treated with genotoxins) from the different blood donors showed a comparable level of basal DNA damage, whereas the contribution of individual susceptibility toward ELF-MF and the tested genotoxic compounds led to differences in the extent of DNA damage observed following exposure to the genotoxins, both in the presence and in the absence of an applied ELF-MF. A 3 mT ELF-MF alone was unable to cause direct primary DNA damage. In leukocytes exposed to ELF-MF and genotoxins, the extent of MNNG-induced DNA damage increased with exposure duration compared to sham-exposed cells. The opposite was observed in cells treated with 4NQO. In this case the extent of 4NQO-induced DNA damage was somewhat reduced in leukocytes exposed to ELF-MF compared to sham-exposed cells. Moreover, in cells exposed to ELF-MF an increased concentration of GSH was always observed, compared to sham-exposed cells. Since following GSH conjugation the genotoxic pattern of MNNG and 4NQO is quite different, an influence of ELF-MF on the activity of the enzyme involved in the synthesis of GSH leading to different activation/deactivation of the model mutagens used was hypothesized to explain the different trends observed in MNNG and 4NQO genotoxic activity in the presence of an applied ELF-MF. The possibility that ELF-MF might interfere with the genotoxic activity of xenobiotics has important implications, since human populations are likely to be exposed to a variety of genotoxic agents concomitantly with exposure to this type of physical agent.

Williams PA, **Ingebretsen RJ**, **Dawson RJ**. 14.6 mT ELF magnetic field exposure yields no DNA breaks in model system Salmonella, but provides evidence of heat stress protection. Bioelectromagnetics. 27(6):445-450, 2006. (NE)

In this study, we demonstrate that common extremely low frequency magnetic field (MF) exposure does not cause DNA breaks in this Salmonella test system. The data does, however, provide evidence that MF exposure induces protection from heat stress. Bacterial cultures were exposed to MF (14.6 mT 60 Hz field, cycled 5 min on, 10 min off for 4 h) and a temperature-matched control. Double- and single-stranded DNA breaks were assayed using a recombination event counter. After MF or control exposure they were grown on indicator plates from which recombination events can be quantified and the frequency of DNA strand breaks deduced. The effect of MF was also monitored using a recombination-deficient mutant (recA). The results showed no significant increase in recombination events and strand breaks due to MF. Evidence of heat stress protection was determined using a cell viability assay that compared the survival rates of MF exposed and control cells after the administration of a 10 min 53 degrees C heat stress. The control cells exhibited nine times more cell mortality than the MF exposed cells. This Salmonella system provides many mutants and genetic tools for further investigation of this phenomenon.

Winker R, **Ivancsits S**, **Pilger A**, **Adlkofer F**, **Rudiger HW**. **Chromosomal damage in human diploid fibroblasts by intermittent exposure to extremely low-frequency electromagnetic fields.** Mutat Res. 585(1-2):43-49, 2005. (E)

Environmental exposure to extremely low-frequency electromagnetic fields (ELF-EMFs) has been implicated in the development of cancer in humans. An important basis for assessing a potential cancer risk due to ELF-EMF exposure is knowledge of biological effects on human cells at the chromosomal level. Therefore, we investigated in the present study the effect of intermittent ELF electromagnetic fields (50 Hz, sinusoidal, 5'field-on/10'field-off, 2-24 h, 1 mT) on the induction of micronuclei (MN) and chromosomal aberrations in cultured human fibroblasts. ELF-EMF radiation resulted in a time-dependent increase of micronuclei, which became significant after 10 h of intermittent exposure at a flux density of 1 mT. After approximately 15 h a constant level of micronuclei of about three times the basal level was reached. In addition, chromosomal aberrations were increased up to 10-fold above basal levels. Our data strongly indicate a clastogenic potential of intermittent low-frequency electromagnetic fields, which may lead to considerable chromosomal damage in dividing cells.

Wolf FI, **Torsello A**, **Tedesco B**, **Fasanella S**, **Boninsegna A**, **D'Ascenzo M**, **Grassi C**, **Azzena GB**, **Cittadini A**. **50-Hz extremely low frequency electromagnetic fields enhance cell proliferation and DNA damage: possible involvement of a redox mechanism.** Biochim Biophys Acta. 1743(1-2):120-129, 2005. (E)

HL-60 leukemia cells, Rat-1 fibroblasts and WI-38 diploid fibroblasts were exposed for 24-72 h to 0.5-1.0-mT 50-Hz extremely low frequency electromagnetic field (ELF-EMF). This treatment induced a dose-dependent increase in the proliferation rate of all cell types, namely about 30% increase of cell proliferation after 72-h exposure to 1.0 mT. This was accompanied by increased percentage of cells in the S-phase after 12- and 48-h exposure. The ability of ELF-EMF to induce DNA damage was also investigated by measuring DNA strand breaks. A dose-dependent increase in DNA damage was observed in all cell lines, with two peaks occurring at 24 and 72 h. A similar pattern of DNA damage was observed by measuring formation of 8-OHdG adducts. The effects of ELF-EMF on cell proliferation and DNA damage were prevented by pretreatment of cells with an antioxidant like alpha-tocopherol, suggesting that redox reactions were involved. Accordingly, Rat-1 fibroblasts that had been exposed to ELF-EMF for 3 or 24 h exhibited a significant increase in dichlorofluorescein-detectable reactive oxygen species, which was blunted by alpha-tocopherol pretreatment. Cells exposed to ELF-EMF and examined as early as 6 h after treatment initiation also exhibited modifications of NF kappa B-related proteins (p65-p50 and I kappa B alpha), which were suggestive of increased formation of p65-p50 or p65-p65 active forms, a process usually attributed to redox reactions. These results suggest that ELF-EMF influence proliferation and DNA damage in both normal and tumor cells through the action of free radical species. This information may be of value for appraising the pathophysiologic consequences of an exposure to ELF-EMF.

Yaguchi H, **Yoshida M**, **Ejima Y**, **Miyakoshi J**. **Effect of high-density extremely low frequency magnetic field on sister chromatid exchanges in mouse m5S cells.** Mutat Res. 440(2):189-194, 1999. (E)

The induction of sister chromatid exchanges (SCEs) was evaluated in the cultured mouse m5S cells after exposure to extremely low frequency magnetic field (ELFMF; 5, 50 and 400 mT). Exposure to 5 mT and 50 mT ELFMF led to a very small increase in the frequency of SCEs, but no significant difference was observed between exposed and unexposed control cells. The cells exposed to 400 mT ELFMF exhibited a significant elevation of the SCE frequencies. There was no significant difference between data from treatments with mitomycin-C (MMC) alone and from combined treatments of MMC plus ELFMF (400 mT) at any MMC concentrations from 4 to 40 nM. These results suggest that exposure to highest-density ELFMF of 400 mT may induce DNA damage, resulting in an elevation of the SCE frequencies. We suppose that there may be a threshold for the elevation of the SCE frequencies, that is at least over the magnetic density of 50 mT.

Yokus B, **Cakir DU**, **Akdag MZ**, **Sert C**, **Mete N**. **Oxidative DNA damage in rats exposed to extremely low frequency electro magnetic fields.** Free Radic Res. 39(3):317-323, 2005. (E)

Extremely low frequency (ELF) electromagnetic field (EMF) is thought to prolong the life of free radicals and can act as a promoter or co-promoter of cancer. 8-hydroxy-2'-deoxyguanosine (8OHdG) is one of the predominant forms of radical-induced lesions to DNA and is a potential tool to asses the cancer risk. We examined the effects of extremely low frequency electro magnetic field (ELF-EMF) (50 Hz, 0.97 mT) on 8OHdG levels in DNA and thiobarbituric acid reactive substances (TBARS) in plasma. To examine the possible time-dependent changes resulting from magnetic field, 8OHdG and TBARS were quantitated at 50 and 100 days. Our results showed that the exposure to ELF-EMF induced oxidative DNA damage and lipid peroxidation (LPO). The 8OHdG levels of exposed group (4.39+/-0.88 and 5.29+/-1.16 8OHdG/dG.10(5), respectively) were significantly higher than sham group at 50 and 100 days (3.02+/-0.63 and 3.46+/-0.38 8OHdG/dG.10(5)) ($p<0.001$, $p<0.001$). The higher TBARS levels were also detected in the exposure group both on 50 and 100 days ($p<0.001$, $p<0.001$). In addition, the extent of DNA damage and LPO would depend on the exposure time ($p<0.05$ and $p<0.05$). Our data may have important implications for the long-term exposure to ELF-EMF which may cause oxidative DNA damage.

Zmyslony M, **Palus J**, **Jajte J**, **Dziubaltowska E**, **Rajkowska E**. **DNA damage in rat lymphocytes treated in vitro with iron cations and exposed to 7 mT magnetic fields (static or 50 Hz).** Mutat Res. 453(1):89-96, 2000. (E)

The present study was undertaken to verify a hypothesis that exposure of the cells to static or 50 Hz magnetic fields (MF) and simultaneous treatment with a known oxidant, ferrous chloride, may affect the oxidative deterioration of DNA molecules.The comet assay was chosen for the assessment of DNA damage. The experiments were performed on isolated rat lymphocytes incubated for 3h in Helmholtz coils at 7 mT static or 50 Hz MF. During MF exposure, part of the cell samples were incubated with 0.01 microM H(2)O(2) and another one with 10 microg/ml FeCl(2,) the rest serving as controls.Lymphocyte exposure to MF at 7 mT did not increase the number of cells with DNA damage in the comet assay. Incubation of lymphocytes with 10 microg/ml FeCl(2) did not produce a detectable damage of DNA either. However, when the FeCl(2)-incubated lymphocytes were simultaneously exposed to 7 mT MF, the number of damaged cells was significantly increased and reached about 20% for static MF and 15% for power frequency MF. In the control samples about 97% of the cells did not have any DNA damage.It is not possible at present to offer a reasonable explanation for the findings of this investigation - the high increase in the number of lymphocytes showing symptoms of DNA damage in the comet assay, following simultaneous exposure to the combination of two non-cytotoxic factors -10 microg/ml FeCl(2) and 7 mT MF. In view of the obtained results we can only hypothesise that under the influence of simultaneous exposure to FeCl(2) and static or 50 Hz MF, the number of reactive oxygen species generated by iron cations may increase substantially. Further studies will be necessary to confirm this hypothesis and define the biological significance of the observed effect.

Zmyslony M, **Palus J**, **Dziubaltowska E**, **Politanski P**, **Mamrot P**, **Rajkowska E**, **Kamedula M**. **Effects of in vitro exposure to power frequency magnetic fields on UV-induced DNA damage of rat lymphocytes.** Bioelectromagnetics. 25(7):560-562, 2004. (E)

The mechanisms of biological effects of 50/60 Hz (power frequency) magnetic fields (MF) are still poorly understood. There are a number of studies indicating that MF affect biochemical processes in which free radicals are involved, such as the biological objects' response to ultraviolet radiation (UVA). Therefore, the present study was aimed to assess the effect of 50 Hz MFs on the oxidative deterioration of DNA in rat lymphocytes irradiated in vitro by UVA. UVA radiation (150 J/m2) was applied for 5 min for all groups and 50 Hz MF (40 microT rms) exposure was applied for some of the groups for 5 or 60 min. The level of DNA damage was assessed using the alkaline comet assay, the fluorescence microscope, and image analysis. It has been found that the 1 h exposure to MF caused an evident increase in all parameters consistent with damaged DNA. This suggest that MF affects the radical pairs generated during the oxidative or enzymatic processes of DNA repair.

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 6

Genetic Effects of Non-Ionizing Electromagnetic Fields

2014 Supplement

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I. INTRODUCTION

The following is an update of information and abstracts on research papers published since 2006/2007 on the genetic effects of nonionizing electromagnetic fields (EMF) in the radiofrequency (RF) and extremely-low frequency (ELF) ranges. Two static magnetic field papers (Jouni et al. 2012; Wang et al., 2009) are also included. Where additional information is relevant, some earlier papers, or papers not specifically related to genetic effects, are also included with citations contained within the discussion below. A list of abstracts, with summary sentences underlined for reader convenience, can be found at the end of this paper.

Analysis of these recent publications shows that there are more papers reporting effects than no effect.

In summary, the new radiofrequency studies report that 65% of genetic studies show effects and 35% do not show effects. **[Effects = 74 (65%) No Effects = 40 (35%)]**

In summary, the new ELF-EMF studies report that 82% of genetic studies show effects and 18% do not show effects **[Effects= 49 (83%) No Effects= 10 (17%)]**

Appendix A has references and abstracts for the RFR literature. Appendix B has references and abstracts for the ELF-EMF literature.

II. GENOTOXIC EFFECTS OF RADIOFREQUENCY RADIATION (RFR) AND OF EXTREMELY LOW FREQUENCY ELECTROMAGNETIC FIELDS (ELF-EMF)

(2007-2014)

The following is an update of information and abstracts on research papers published since 2006/2007 on the genetic effects of nonionizing electromagnetic fields (EMF) in the radiofrequency (RF) and extremely-low frequency (ELF) ranges. Two static magnetic field papers (Jouni et al. 2012; Wang et al., 2009) are also included. Where additional information is relevant, some earlier papers, or papers not specifically related to genetic effects, are also included with citations contained within the discussion below. A list of abstracts, with summary sentences underlined for reader convenience, can be found at the end of this paper.

Analysis of these recent publications shows that there are more papers reporting effects than no effect. With E representing a biological effect, and NE representing no biological effects, the recent literature finds RFR-genetic effects at: E=74 publications (65%); NE=40 publications (35%); and ELF-genetic effects at: E=49 (83%); NE=10 (17%).

Discussion

1. The effects of both RF and ELF fields are very similar. This is surprising because the energies carried by these EMFs are billions of folds different. An explanation for similar genetic effects has been provided by a recent paper by Blank and Goodman (Blank M, Goodman R. DNA is a fractal antenna in electromagnetic fields. Int. J. Radiat. Biol. 87(4):409-415, 2011) in which they stated that '…the wide frequency range of interaction with EMF is the functional characteristic of a fractal antenna, and DNA appears to possess the two structural characteristics of fractal antennas, electronic conduction and self symmetry.' However, similarities in effects between ELF and RF fields have also been reported in studies of other physiological processes, e.g., neurochemical and behavioral effects (Cf. Lai, H., Carino, M.A., Horita, A. and Guy, A.W. Opioid receptor subtypes that mediate a microwave-induced decrease in central cholinergic activity in the rat. Bioelectromagnetics 13:237-246, 1992; Lai, H. and Carino, M.A. Intracerebroventricular injections of mu and delta-opiate receptor antagonists block 60-Hz magnetic field-induced decreases in cholinergic activity in the frontal cortex and hippocampus of the rat. Bioelectromagnetics 19:433-437, 1998; Lai, H., Carino, M.A. and Ushijima, I. Acute exposure to a 60 Hz magnetic field affects rats' performance in the water maze. Bioelectromagnetics 19:117-122, 1998; Wang, B.M. and Lai, H. Acute exposure to pulsed 2450-MHz microwaves affects water maze learning in the rat. Bioelectromagnetics 21:52-56, 2000.) Thus, there is a basic interaction mechanism of biological tissues with electromagnetic fields that is independent of frequency.
   Many studies have implicated the involvement of free radical processes in the genetic effects of EMF: ELF-EMF (Butdak et al., 2012; Jouni et al., 2012; Luukkonen et al., 2014; Tiwari et al.,2014); RFR (Agarwal et al., 2009; Atasoy et al., 2012; Burlaka et al., 2013; Campisi et al., 2010; De Iuliis et al., 2009; Esmekaya et al., 2011; Ferreira et al., 2006; Gajski and Garaj-Vrhovac, 2009; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Khalil et al., 2012; Kumar et al., 2010; Liu et al., 2013a,b; Luukkonan et al., 2009; Tomruk et al., 2010; Tkalec et al., 2013; Wu et al., 2008; Xu et al., 2010; Yao et al., 2003). Increase in free radical activity and changes in enzymes involved in cellular oxidative processes are the most consistent effects observed in cells and animals after EMF exposure. However, they are reports indicating that EMF could induce genetic effects without the involvement of free radicals (ELF- Alcaraz et al., 2013; RFR- Ferreira et al., 2006; Furtado-Filho et al., 2013) and increase in free radical after EMF exposure did not lead to genetic effects (Frahm et al., 2006). There are at least a couple of hundred published papers on the effects of EMF exposure on cellular oxidative processes. Many biological effects of EMF can be explained by intracellular changes in oxidative status, including the genetic effects reported in this review.

2. An important observation of the studies is that EMF can interact with other entities and synergistically cause genetic effects. These entities include: ELF-EMF- cisplastin (Buldak et al., 2012; El-Bialy et al., 2013), bleomycin (Cho et al., 2007), gadolinium (Cho et al., 2014); hydrogen peroxide and methyl methane sulfonate (Koyama et al., 2008), menadione (Luukkonan et al., 2011, 2014; Markkanen et al., 2008), ionizing radiation (Mairs et al., 2007; Journi et al., 2012 Yoon et al., 2014); RFR- chemical

mutagens (Baohong et al., 2005), clastogens (Kim et al., 2008), x-rays (Manti et al., 2008), ultraviolet ray (Baohong et al., 2007), aphidicolin (Tiwari et al., 2008), picrotoxin (López-Martín et al., 2009), doxorubicin (Zhijian et al., 2010), and incoherent electromagnetic noise (Wu et al., 2008; Yao et al., 2008). Most of the compounds that interact with EMF are mutagens. This is important because in real life situations, a person is usually exposed to many different environmental factors simultaneously. Synergism of these factors with EMF should be considered more seriously.

3. Several long term/repeated exposure papers are included in this update: ELF-EMF (Borhani et al., 2011; Cuccurazzu et al., 2010; Erdal et al., 2007; Fedrowitz and Loscher, 2012; Mariucci et al., 2010; Panagopoulous et al., 2013; Udroiu et al., 2006), and RFR (Asasoy et al., 2012; Atli Serkeroglu et al., 2013; Burlaka et al., 2013; Chavdoula et al., 2010; Deshmukh et al., 2013; Ferreira et al., 2006; Garaj-Vrhovac et al., 2011; Guler et al., 2010, 2012; Kesari and Behari, 2009; Kesari et al., 2010; Lakshmi et al., 2010; Paulraj and Behari, 2006; Tomruk et al., 2010; Yan et al., 2008). These data are important in the understanding of the biological effects of EMF exposure in real life situation, since human environmental EMF exposure is both chronic and intermittent. Within these long-term exposure studies, there are several that investigated the effect of EMF exposure on developing animals (ELF-EMF: Borhani et al., 2011; Cuccurazzu et al., 2010; Panagopoulous et al., 2013; Udroiu et al., 2006, RFR: Burlaka et al., 2013; Ferreira et al., 2006; Guler et al., 2010, 2012; Serkeroglu et al., 2013; Tomruk et al., 2010; Zalata et al., In press). Data of effects of EMF exposure on growth and development of young animals are urgently needed. There are several studies indicating that RFR may affect reproduction, particularly with effects on sperm physiology and DNA (Agarwal et al., 2009; Atasoy et al., 2012; Avendano et al., 2012; Chavdoula et al., 2010; de Iuliis et al., 2009; Liu et al., 2013b; Panagopoulous et al., 2007). Similar effects of ELF-EMF on sperm have also been reported, e.g., Hong R, Zhang Y, Liu Y, Weng EQ. Effects of extremely low frequency electromagnetic fields on DNA of testicular cells and sperm chromatin structure in mice. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 23(6):414-417, 2005; Iorio R, Scrimaglio R, Rantucci E, Delle Monache S, Di Gaetano A, Finetti N, Francavilla F, Santucci R, Tettamanti E, Colonna R. A preliminary study of oscillating electromagnetic field effects on human spermatozoon motility. Bioelectromagnetics. 28(1):72-75, 2007; Iorio R, Delle Monache S, Bennato F, Di Bartolomeo C, Scrimaglio R, Cinque B, Colonna RC. Involvement of mitochondrial activity in mediating ELF-EMF stimulatory effect on human sperm motility. Bioelectromagnetics. 32(1):15-27, 2011.

4. Another area that needs more research is the biological effects of low-intensity exposure. This is particularly true for ELF-EMF, since intensities of ELF-EMF in the environment are in microtesla (µT) levels. There are many studies on biological effects of low-intensity RFR (see Table 1 in Levitt, B.B. and Lai, H. Biological effects from exposure to electromagnetic radiation emitted by cell tower base stations and other antenna arrays. Environ. Rev. 18:369-395, 2010.) However, most cell and animal studies in ELF-EMF used fields in the millitesla (mT) level. Exceptions are the study of Sarimov et al. (2011) listed below in the reference section and the study of de Bruyn and de Jager (2010) (de Bruyn L and de Jager L. Effect of long-term exposure to a randomly varied 50

Hz power frequency magnetic field on the fertility of the mouse. Electromag. Biol. Med. 29(1-2):52-61, 2010).

5. Two other important findings of these recent studies are that the effects of EMF are shown to be waveform specific and cell-type specific. Regarding waveform specificity, Campisi et al. (2010) reported increases in free radical activity and DNA fragmentation in brain cells after acute exposure to a 50-Hz amplitude-modulated 900-MHz RFR, whereas a continuous-wave 9000-MHz field produced no effect. Franzellitti et al. (2010) showed increased DNA strand breaks in trophoblasts after exposure to a 217-Hz modulated 1.8 GHz-RFR, but a continuous-wave field of the same carrier frequency was without effect. Tkalec et al (2013) reported that AM-modulated (1 KHz sinusoidal) 900-MHz RFR is more potent than non-modulated field in causing DNA damage in coelomocytes of exposed earthworms. Luukkonen et al. (2009) reported a continuous-wave 872-MHz RFR increased chemically-induced DNA strand breaks and free radicals in human neuroblastoma cells, whereas a GSM-modulated 872-MHz field had no significant effect. Zhang et al. (2008) found that gene expression in rat neurons is more sensitive to intermittent than continuous exposure to a 1.8 GHz-RFR. López-Martín et al. (2009) found that GSM and unmodulated RFR caused different effects on c-Fos gene expression in the rat brain. Regarding cell-type specificity, Nylund and Leszczynski (2006) and Remondini et al. (2006) reported different patterns of gene expression in different types of cells after exposure to RFR. Zhao et al. (2007) found than neurons are more sensitive to a 1.9 GHz cell phone radiation than astrocytes. Schwarz et al. (2008) reported DNA strand breaks and micronucleus formation in human fibroblasts, but not in lymphocytes, after exposure to a 1950-MHz UMTS field. Furthermore, Xu et al (2013) found DNA damages in some cell types and not in others after exposure to 1800-MHz RFR. Valbonesi et at. (2014) reported that HSP70 expression and MAPK signaling pathways in PC12 cells were affected by GSM-217 Hz signal and not by CW or GSM-talk signals. In ELF-EM research, Giorgi et al. (2011) found that DNA transposition in E. coli was *decreased* after exposure to a sinusoidal magnetic field and *increased* after exposure to a pulsed magnetic field. Kim et al. (2012) described DNA strand breaks in human fibroblasts after exposure to ELF magnetic field. They found that the pattern of changes depended on the eddy current and Lorentz force in the field. Nahab et al. (2007) reported that a square-continuous ELF magnetic field was more effective than sinusoidal-continuous or pulsed field in inducing sister chromatid exchange in human lymphocytes. These findings underscore the complicity of interaction of EMF with biological tissues and may partially explain why effects were observed in some studies and not others. It is essential to understand why and how certain wave-characteristics of an EMF are more effective than other characteristics in causing biological effects, and why certain types of cells are more susceptible to the effect of EMF? That there are different biological effects elicited by different EMF wave characteristics is critical proof for the existence of nonthermal effects.

6. Many biological/health effects have been reported in cells and animals after exposure to EMFs in both the ELF and RF ranges. (Sixty-five percent of the RFR papers and 82% of the ELF-EMF papers in the publication list below reported effects.) It is highly dishonest for a scientist to summarily deny the existence of biological effects of EMF. A

biological effect of EMF can be detrimental to health, but can also be turned into a beneficial means for the treatment of human diseases. Denying any effects hampers the development of electromagnetic treatments for diseases. Examples of possible clinical uses of EMF are: Alzheimer's disease (Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, Runfeldt M, Wang L, Zhang G, Sava V, Tan J, Cao C. Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. J Alzheimers Dis. 19(1):191-210, 2010); Parkinson's disease (Wang Z, Che PL, Du J, Ha B, Yarema KJ. Static magnetic field exposure reproduces cellular effects of the Parkinson's disease drug candidate ZM241385. PLoS One. 5(11):e13883, 2010); bone regeneration (Lee HM, Kwon UH, Kim H, Kim HJ, Kim B, Park JO, Moon ES, Moon SH. Pulsed eltromagnetic field stimulates cellular proliferation in human intervertebral disc cells. Yonsei Med. J. 51(6):954-959, 2010); cancer treatment (Costa FP, de Oliveira AC, Meirelles R, Machado MC, Zanesco T, Surjan R, Chammas MC, de Souza Rocha M, Morgan D, Cantor A, Zimmerman J, Brezovich I, Kuster N, Barbault A, Pasche B. Treatment of advanced hepatocellular carcinoma with very low levels of amplitude-modulated electromagnetic fields. Br. J. Cancer. 105(5):640-648, 2011), and tissue regeneration (Gaetani R, Ledda M, Barile L, Chimenti I, De Carlo F, Forte E, Ionta V, Giuliani L, D'Emilia E, Frati G, Miraldi F, Pozzi D, Messina E, Grimaldi S, Giacomello A, Lisi A. Differentiation of human adult cardiac stem cells exposed to extremely low-frequency electromagnetic fields. Cardiovasc. Res. 82(3):411-420, 2009).

7. It must be pointed out that, consistent with previous research, not very much of the cellular and animal genetic research data directly indicate that EMF (both RF and ELF EMF) is a carcinogen. However, the data show that EMF can possibly alter genetic functions and thus it is advisable that one should limit one's exposure to EMF.

APPENDIX A - ABSTRACTS ON GENETIC EFFECTS OF RADIOFREQUENCY AND CELL PHONE RADIATION (2007-2014)

Below is a key to abbreviations used throughout the following list of abstracts for recent papers published since 2006 and serve as my comments to help the reader quickly identify the significance of each work. The summary sentences by each author are underlined. The list is divided into RF effects papers, and ELF effects papers.

(E- effect observed; NE- no effect observed) (LE- long term exposure; GT- genotoxic effect, e.g., DNA damage, micronucleus formation, chromosome alterations; GE- gene expression; HU- human study; OX- oxidative effects, i.e., involvement of free radicals and oxidative enzymes; IA- interaction with other factors to cause genetic effects; DE- effects on developing animals; RP- reproduction, e.g., sperm damage; EH- compared with electro-hypersensitive subjects; WS- waveform specific effect, e.g., modulation and frequency; CS- cell type specific effect).

(E) Agarwal A, Desai NR, Makker K, Varghese A, Mouradi R, Sabanegh E, Sharma R. Effects of radiofrequency electromagnetic waves (RF-EMW) from cellular phones on human ejaculated semen: an in vitro pilot study. Fertil Steril 92 1318-1325, 2009. (GT, RP, OX)

OBJECTIVE: To evaluate effects of cellular phone radiofrequency electromagnetic waves (RF-EMW) during talk mode on unprocessed (neat) ejaculated human semen. DESIGN: Prospective pilot study. SETTING: Center for reproductive medicine laboratory in tertiary hospital setting. SAMPLES: Neat semen samples from normal healthy donors (n = 23) and infertile patients (n = 9). INTERVENTION(S): After liquefaction, neat semen samples were divided into two aliquots. One aliquot (experimental) from each patient was exposed to cellular phone radiation (in talk mode) for 1 h, and the second aliquot (unexposed) served as the control sample under identical conditions. MAIN OUTCOME MEASURE(S): Evaluation of sperm parameters (motility, viability), reactive oxygen species (ROS), total antioxidant capacity (TAC) of semen, ROS-TAC score, and sperm DNA damage. RESULT(S): Samples exposed to RF-EMW showed a significant decrease in sperm motility and viability, increase in ROS level, and decrease in ROS-TAC score. Levels of TAC and DNA damage showed no significant differences from the unexposed group. CONCLUSION(S): Radiofrequency electromagnetic waves emitted from cell phones may lead to oxidative stress in human semen. We speculate that keeping the cell phone in a trouser pocket in talk mode may negatively affect spermatozoa and impair male fertility.

(E) Atasoy HI, Gunal MY, Atasoy P, Elgun S, Bugdayci G. Immunohistopathologic demonstration of deleterious effects on growing rat testes of radiofrequency waves emitted from conventional Wi-Fi devices.J Pediatr Urol. 2012 Mar 30. [Epub ahead of print] (GT, OX, LE, RP)

OBJECTIVE: To investigate effects on rat testes of radiofrequency radiation emitted from indoor Wi-Fi Internet access devices using 802.11.g wireless standards. METHODS: Ten Wistar albino male rats were divided into experimental and control groups, with five rats per group. Standard wireless gateways communicating at 2.437 GHz were used as radiofrequency wave sources. The experimental group was exposed to radiofrequency energy for 24 h a day for 20 weeks. The rats were sacrificed at the end of the study. Intracardiac blood was sampled for serum 8-hydroxy-2'-deoxyguanosine levels. Testes were removed and examined histologically and immunohistochemically. Testis tissues were analyzed for malondialdehyde levels and prooxidant-antioxidant enzyme activities. RESULTS: We observed significant increases in serum 8-hydroxy-2'-deoxyguanosine levels and 8-hydroxyguanosine staining in the testes of the experimental group indicating DNA damage due to exposure ($p < 0.05$). We also found decreased levels of catalase and glutathione peroxidase activity in the experimental group, which may have been due to radiofrequency effects on enzyme activity ($p < 0.05$). CONCLUSIONS: These findings raise questions about the safety of radiofrequency exposure from Wi-Fi Internet access devices for growing organisms of reproductive age, with a potential effect on both fertility and the integrity of germ cells.

(E) Atlı Şekeroğlu Z, Akar A, Sekeroğlu V. Evaluation of the cytogenotoxic damage in immature and mature rats exposed to 900 MHz radio frequency electromagnetic fields. Int J Radiat Biol. 89(11):985-992, 2013. [Epub ahead of print] (GT, DE, LE)

Abstract Purpose: One of the most important issues regarding radio frequency electromagnetic fields (RF-EMF) is their effect on genetic material. Therefore, we investigated the cytogenotoxic effects of 900 MHz radio frequency electromagnetic fields (RF-EMF) and the effect of a recovery period after exposure to RF-EMF on bone marrow cells of immature and mature rats. Materials and methods: The immature and mature rats in treatment groups were exposed to RF-EMF for 2 h/day for 45 days. Average electrical field values for immature and mature rats were 28.1±4.8 V/m and 20.0±3.2 V/m, respectively. Whole-body specific absorption rate (SAR) values for immature and mature rats were in the range of 0.38-0.78 W/kg, and 0.31-0.52 W/kg during the 45 days, respectively. Two recovery groups were kept for 15 days after RF-EMF exposure. Results: Significant differences were observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCE) in all treatment and recovery groups. The cytogenotoxic damage in immature rats was statistically higher than the mature rats. The recovery period did not reduce the damage to the same extent as the corresponding control groups. Conclusions: The exposure of RF-EMF leads to cytotoxic and genotoxic damage in immature and mature rats. More sensitive studies are required to elucidate the possible carcinogenic risk of EMF exposure in humans, especially children.

(E) Avendaño C, Mata A, Sanchez Sarmiento CA, Doncel GF. Use of laptop computers connected to internet through Wi-Fi decreases human sperm motility and increases sperm DNA fragmentation. FertilSteril 97:39-45, 2012. (GT, RP)

OBJECTIVE: To evaluate the effects of laptop computers connected to local area networks wirelessly (Wi-Fi) on human spermatozoa. DESIGN: Prospective in vitro study. SETTING: Center for reproductive medicine. PATIENT(S): Semen samples from 29 healthy donors. INTERVENTION(S): Motile sperm were selected by swim up. Each sperm suspension was divided into two aliquots. One sperm aliquot (experimental) from each patient was exposed to an internet-connected laptop by Wi-Fi for 4 hours, whereas the second aliquot (unexposed) was used as control, incubated under identical conditions without being exposed to the laptop. MAIN OUTCOME MEASURE(S): Evaluation of sperm motility, viability, and DNA fragmentation. RESULT(S): Donor sperm samples, mostly normozoospermic, exposed ex vivo during 4 hours to a wireless internet-connected laptop showed a significant decrease in progressive sperm motility and an increase in sperm DNA fragmentation. Levels of dead sperm showed no significant differences between the two groups. CONCLUSION(S): To our knowledge, this is the first study to evaluate the direct impact of laptop use on human spermatozoa. Ex vivo exposure of human spermatozoa to a wireless internet-connected laptop decreased motility and induced DNA fragmentation by a nonthermal effect. We speculate that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility. Further in vitro and in vivo studies are needed to prove this contention.

(E) Baohong Wang, Jiliang H, Lifen J, Deqiang L, Wei Z, Jianlin L, Hongping D. Studying the synergistic damage effects induced by 1.8 GHz radiofrequency field radiation (RFR) with four chemical mutagens on human lymphocyte DNA using comet assay in vitro. Mutat Res 578:149-57, 2005. (GT, IA)

The aim of this investigation was to study the synergistic DNA damage effects in human lymphocytes induced by 1.8GHz radiofrequency field radiation (RFR, SAR of 3W/kg) with four chemical mutagens, i.e. mitomycin C (MMC, DNA crosslinker), bleomycin (BLM, radiomimetic agent), methyl methanesulfonate (MMS, alkylating agent), and 4-nitroquinoline-1-oxide (4NQO, UV-mimetic agent). The DNA damage of lymphocytes exposed to RFR and/or with chemical mutagens was detected at two incubation time (0 or 21h) after treatment with comet assay in vitro. Three combinative exposure ways were used. Cells were exposed to RFR and chemical mutagens for 2 and 3h, respectively. Tail length (TL) and tail moment (TM) were utilized as DNA damage indexes. The results showed no difference of DNA damage indexes between RFR group and control group at 0 and 21h incubation after exposure (P>0.05). There were significant difference of DNA damage indexes between MMC group and RFR+MMC co-exposure group at 0 and 21h incubation after treatment (P<0.01). Also the significant difference of DNA damage indexes between 4NQO group and RFR+4NQO co-exposure group at 0 and 21h incubation after treatment was observed (P<0.05 or P<0.01). The DNA damage in RFR+BLM co-exposure groups and RFR+MMS co-exposure groups was not significantly increased, as compared with corresponding BLM and MMS groups (P>0.05). The experimental results indicated 1.8GHz RFR (SAR, 3W/kg) for 2h did not induce the human lymphocyte DNA damage effects in vitro, but could enhance the human lymphocyte DNA damage effects induced by MMC and 4NQO. The synergistic DNA damage effects of 1.8GHz RFR with BLM or MMS were not obvious.

(E) Baohong W, Lifen J, Lanjuan L, Jianlin L, Deqiang L, Wei Z, Jiliang H.Evaluating the combinative effects on human lymphocyte DNA damage induced by ultraviolet ray C plus 1.8GHz microwaves using comet assay in vitro. Toxicology. 232(3):311-316, 2007. (GT, IA)

The objective of this study was to observe whether 1.8GHz microwaves (MW) (SAR, 3 W/kg) exposure can influence human lymphocyte DNA damage induced by ultraviolet ray C (UVC). The lymphocytes, which were from three young healthy donors, were exposed to 254 nm UVC at the doses of 0.25, 0.5, 0.75, 1.0, 1.5 and 2.0 J m(-2), respectively. The lymphocytes were irradiated by 1.8GHz MW (SAR, 3 W/kg) for 0, 1.5 and 4 h. The combinative exposure of UVC plus MW was conducted. The treated cells were incubated for 0, 1.5 and 4 h. Finally, comet assay was used to measure DNA damage of above treated lymphocytes. The results indicated that the difference of DNA damage induced between MW group and control group was not significant (P>0.05). The MTLs induced by UVC were 1.71+/-0.09, 2.02+/-0.08, 2.27+/-0.17, 2.27+/-0.06, 2.25+/-0.12, 2.24+/-0.11 microm, respectively, which were significantly higher than that (0.96+/-0.05 microm) of control (P<0.01). MTLs of some sub-groups in combinative exposure groups at 1.5-h incubation were significantly lower that those of corresponding UVC sub-groups (P<0.01 or P<0.05). However, MTLs of some sub-groups in combinative exposure groups at 4-h incubation were significantly higher that those of corresponding UVC sub-groups (P<0.01 or P<0.05). In this experiment it was found that 1.8GHz (SAR, 3 W/kg) MW exposure for 1.5 and 4 h did not enhance significantly human lymphocyte DNA damage, but could reduce and increase DNA damage of human lymphocytes induced by UVC at 1.5-h and 4-h incubation, respectively.

(E) Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LO, Persson BR, Selivanova G, Harms-Ringdahl M. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. Bioelectromagnetics 26:173-184, 2005. (GT, EH)

We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power frequency magnetic field (50 Hz, 15 muT peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock.

(E) Belyaev IY, Koch CB, Terenius O, Roxstrom-Lindquist K, Malmgren LO, H Sommer W, Salford LG, Persson BR. Exposure of rat brain to 915 MHz GSM microwaves induces changes in gene expression but not double stranded DNA breaks or effects on chromatin conformation. Bioelectromagnetics 27:295-306, 2006. (GE)

We investigated whether exposure of rat brain to microwaves (MWs) of global system for mobile communication (GSM) induces DNA breaks, changes in chromatin conformation and in gene expression. An exposure installation was used based on a test mobile phone employing a GSM signal at 915 MHz, all standard modulations included, output power level in pulses 2 W, specific absorption rate (SAR) 0.4 mW/g. Rats were exposed or sham exposed to MWs during 2 h. After exposure, cell suspensions were prepared from brain samples, as well as from spleen and thymus. For analysis of gene expression patterns, total RNA was extracted from cerebellum. Changes in chromatin conformation, which are indicative of stress response and genotoxic effects, were measured by the method of anomalous viscosity time dependencies (AVTD). DNA double strand breaks (DSBs) were analyzed by pulsed-field gel electrophoresis (PFGE). Effects of MW exposure were observed on neither conformation of chromatin nor DNA DSBs. Gene expression profiles were obtained by Affymetrix U34 GeneChips representing 8800 rat genes and analyzed with the Affymetrix Microarray Suite (MAS) 5.0 software. In cerebellum from all exposed animals, 11 genes were upregulated in a range of 1.34-2.74 fold and one gene was downregulated 0.48-fold ($P < .0025$). The induced genes encode proteins with diverse functions including neurotransmitter regulation, blood-brain barrier (BBB), and melatonin production. The data shows that GSM MWs at 915 MHz did not induce PFGE-detectable DNA double stranded breaks or changes in chromatin conformation, but affected expression of genes in rat brain cells

(E) Belyaev IY, Markovà E, Hillert L, Malmgren LO, Persson BR. Microwaves from UMTS/GSM mobile phones induce long-lasting inhibition of 53BP1/gamma-H2AX DNA repair foci in human lymphocytes. Bioelectromagnetics 30:129-41, 2009. (GT, EH)

We have recently described frequency-dependent effects of mobile phone microwaves (MWs) of global system for mobile communication (GSM) on human lymphocytes from persons reporting hypersensitivity to electromagnetic fields and healthy persons. Contrary to GSM, universal global telecommunications system (UMTS) mobile phones emit wide-band MW signals. Hypothetically, UMTS MWs may result in higher biological effects compared to GSM signal because of eventual "effective" frequencies within the wideband. Here, we report for the first time that UMTS MWs affect chromatin and inhibit formation of DNA double-strand breaks co-localizing 53BP1/gamma-H2AX DNA repair foci in human lymphocytes from hypersensitive and healthy persons and confirm that effects of GSM MWs depend on carrier frequency. Remarkably, the effects of MWs on 53BP1/gamma-H2AX foci persisted up to 72 h following exposure of cells, even longer than the stress response following heat shock. The data are in line with the hypothesis that the type of signal, UMTS MWs, may have higher biological efficiency and possibly larger health risk effects compared to GSM radiation emissions. No significant differences in effects between groups of healthy and hypersensitive subjects were observed, except for the effects of UMTS MWs and GSM-915 MHz MWs on the formation of the DNA repair foci, which were different for hypersensitive ($P < 0.02$[53BP1]//0.01[gamma-H2AX]) but not for control subjects ($P > 0.05$). The non-parametric statistics used here did not indicate specificity of the differences revealed between the effects of GSM and UMTS MWs on cells from hypersensitive subjects and more data are needed to study the nature of these differences.

(NE) Bourthoumieu S, Joubert V, Marin B, Collin A, Leveque P, Terro F, Yardin C. Cytogenetic studies in human cells exposed in vitro to GSM-900 MHz radiofrequency radiation using R-banded karyotyping. Radiat Res 174:712-718, 2010. (GT)

It is important to determine the possible effects of exposure to radiofrequency (RF) radiation on the genetic material of cells since damage to the DNA of somatic cells may be linked to cancer development or cell death and damage to germ cells may lead to genetic damage in next and subsequent generations. The objective of this study was to investigate whether exposure to radiofrequency radiation similar to that emitted by mobile phones of second-generation standard Global System for Mobile Communication (GSM) induces genotoxic effects in cultured human cells. The cytogenetic effects of GSM-900 MHz (GSM-900) RF radiation were investigated using R-banded karyotyping after in vitro exposure of human cells (amniotic cells) for 24 h. The average specific absorption rate (SAR) was 0.25 W/kg. The exposures were carried out in wire-patch cells (WPCs) under strictly controlled conditions of temperature. The genotoxic effect was assessed immediately or 24 h after exposure using four different samples. One hundred metaphase cells were analyzed per assay. Positive controls were provided by using bleomycin. We found no direct cytogenetic effects of GSM-900 either 0 h or 24 h after exposure. To the best of our knowledge, our work is the first to study genotoxicity using complete R-banded karyotyping, which allows visualizing all the chromosomal rearrangements, either numerical or structural.

(NE) Bourthoumieu S, Terro F, Leveque P, Collin A, Joubert V, Yardin C. Aneuploidy studies in human cells exposed in vitro to GSM-900 MHz radiofrequency radiation using FISH. Int J Radiat Biol 87:400-408, 2011. (GT)

PURPOSE: Since previous research found an increase in the rate of aneuploidies in human lymphocytes exposed to radiofrequencies, it seems important to perform further studies. The objective of this study was then to investigate whether the exposure to RF (radiofrequency) radiation similar to that emitted by mobile phones of a second generation standard, i.e., Global System for Mobile communication (GSM) may induce aneuploidy in cultured human cells. MATERIALS AND METHODS: The potential induction of genomic instability by GSM-900 MHz radiofrequency (GSM-900) was investigated after in vitro exposure of human amniotic cells for 24 h to average-specific absorption rates (SAR) of 0.25, 1, 2 and 4 W/kg in the temperature range of 36.3-39.7°C. The exposures were carried out in a wire-patch cell (WPC). The rate of aneuploidy of chromosomes 11 and 17 was determined by interphase FISH (Fluorescence In Situ Hybridisation) immediately after independent exposure of three different donors for 24 h. At least 100 interphase cells were analysed per assay. RESULTS: No significant change in the rate of aneuploidy of chromosomes 11 and 17 was found following exposure to GSM-900 for 24 h at average SAR up to 4 W/kg. CONCLUSION: Our study did not show any in vitro aneuploidogenic effect of GSM using FISH and is not in agreement with the results of previous research.

(NE) Bourthoumieu S, Magnaudeix A, Terro F, Leveque P, Collin A, Yardin C. Study of p53 expression and post-transcriptional modifications after GSM-900 radiofrequency exposure of human amniotic cells. Bioelectromagnetics. 2012 Jul 5. doi: 10.1002/bem.21744. [Epub ahead of print] (GE)

The potential effects of radiofrequency (RF) exposure on the genetic material of cells are very important to determine since genome instability of somatic cells may be linked to cancer development. In response to genetic damage, the p53 protein is activated and can induce cell cycle arrest allowing more time for DNA repair or elimination of damaged cells through apoptosis. The objective of this study was to investigate whether the exposure to RF electromagnetic fields, similar to those emitted by mobile phones of the second generation standard, Global System for Mobile Communications (GSM), may induce expression of the p53 protein and its activation by post-translational modifications in cultured human cells. The potential induction of p53 expression and activation by GSM-900 was investigated after in vitro exposure of human amniotic cells for 24 h to average specific absorption rates (SARs) of 0.25, 1, 2, and 4 W/kg in the temperature range of 36.3-39.7 °C. The exposures were carried out using a wire-patch cell (WPC) under strictly controlled conditions of temperature. Expression and activation of p53 by phosphorylation at serine 15 and 37 were studied using Western blot assay immediately after three independent exposures of cell cultures provided from three different donors. Bleomycin-exposed cells were used as a positive control. According to our results, no significant changes in the expression and activation of the p53 protein by phosphorylation at serine 15 and 37 were found following exposure to GSM-900 for 24 h at average SARs up to 4 W/kg in human embryonic cells.

(E) Burlaka A, Tsybulin O, Sidorik E, Lukin S, Polishuk V, Tsehmistrenko S, Yakymenko I. Overproduction of free radical species in embryonal cells exposed to low intensity radiofrequency radiation. Exp Oncol. 35(3):219-225, 2013. (GT, LE, DE, OX)

Aim: Long-term exposure of humans to low intensity radiofrequency electromagnetic radiation (RF-EMR) leads to a statistically significant increase in tumor incidence. Mechanisms of such the effects are unclear, but features of oxidative stress in living cells under RF-EMR exposure were previously reported. Our study aims to assess a production of initial free radical species, which lead to oxidative stress in the cell. Materials and Methods: Embryos of Japanese quails were exposed in ovo to extremely low intensity RF-EMR of GSM 900 MHz (0.25 µW/cm2) during 158-360 h discontinuously (48 c - ON, 12 c - OFF) before and in the initial stages of development. The levels of superoxide (O2·-), nitrogen oxide (NO·), thiobarbituric acid reactive substances (TBARS), 8-oxo-2'-deoxyguanosine (8-oxo-dG) and antioxidant enzymes' activities were assessed in cells/tissues of 38-h, 5- and 10-day RF-EMR exposed and unexposed embryos. Results: The exposure resulted in a significant persistent overproduction of superoxide and nitrogen oxide in embryo cells during all period of analyses. As a result, significantly increased levels of TBARS and 8-oxo-dG followed by significantly decreased levels of superoxide dismutase and catalase activities were developed in the exposed embryo cells. Conclusion: Exposure of developing quail embryos to extremely low intensity RF-EMR of GSM 900 MHz during at least one hundred and fifty-eight hours leads to a significant overproduction of free radicals/reactive oxygen species and oxidative damage of DNA in embryo cells. These oxidative changes may lead to pathologies up to oncogenic transformation of cells.

(E) Buttiglione M, Roca L, Montemurno E, Vitiello F, Capozzi V, Cibelli G. Radiofrequency radiation (900 MHz) induces Egr-1 gene expression and affects cell-cycle control in human neuroblastoma cells. J Cell Physiol. 213(3):759-767, 2007. (GE)

Many environmental signals, including ionizing radiation and UV rays, induce activation of Egr-1 gene, thus affecting cell growth and apoptosis. The paucity and the controversial knowledge about the effect of electromagnetic fields (EMF) exposure of nerve cells prompted us to investigate the bioeffects of radiofrequency (RF) radiation on SH-SY5Y neuroblastoma cells. The effect of a modulated RF field of 900 MHz, generated by a wire patch cell (WPC) antenna exposure system on Egr-1 gene expression, was studied as a function of time. Short-term exposures induced a transient increase in Egr-1 mRNA level paralleled with activation of the MAPK subtypes ERK1/2 and SAPK/JNK. The effects of RF radiations on cell growth rate and apoptosis were also studied. Exposure to RF radiation had an anti-proliferative activity in SH-SY5Y cells with a significant effect observed at 24 h. RF radiation impaired cell cycle progression, reaching a significant G2-M arrest. In addition, the appearance of the sub-G1 peak, a hallmark of apoptosis, was highlighted after a 24-h exposure, together with a significant decrease in mRNA levels of Bcl-2 and survivin genes, both interfering with signaling between G2-M arrest and apoptosis. Our results provide evidence that exposure to a 900 MHz-modulated RF radiation affect both Egr-1 gene expression and cell regulatory functions, involving apoptosis inhibitors like Bcl-2 and survivin, thus providing important insights into a potentially broad mechanism for controlling in vitro cell viability.

(E) Cam ST, Seyhan N. Single-strand DNA breaks in human hair root cells exposed to mobile phone radiation. Int J Radiat Biol 88(5):420-424, 2012 (GT, HU)

Purpose: To analyze the short term effects of radiofrequency radiation (RFR) exposure on genomic deoxyribonucleic acid (DNA) of human hair root cells. Subjects and methods: Hair samples were collected from 8 healthy human subjects immediately before and after using a 900-MHz GSM (Global System for Mobile Communications) mobile phone for 15 and 30 minutes. Single-strand DNA breaks of hair root cells from the samples were determined using the 'comet assay'. Results: The data showed that talking on a mobile phone for 15 or 30 minutes significantly increased (p< .05) single-strand DNA breaks in cells of hair roots close to the phone. Comparing the 15-min and 30-min data using the paired t-test also showed that significantly more damages resulted after 30 minutes than after 15 minutes of phone use. Conclusions: A short-term exposure (15 and 30 minutes) to RFR (900-MHz) from a mobile phone caused a significant increase in DNA single-strand breaks in human hair root cells located around the ear which is used for the phone calls.

(E) Campisi A, Gulino M, Acquaviva R, Bellia P, Raciti G, Grasso R, Musumeci F, Vanella A, Triglia A. Reactive oxygen species levels and DNA fragmentation on astrocytes in primary culture after acute exposure to low intensity microwave electromagnetic field. Neurosci Lett 473:52-55. 2010. (GT, OX, WS)

The exposure of primary rat neocortical astroglial cell cultures to acute electromagnetic fields (EMF) in the microwave range was studied. Differentiated astroglial cell cultures at 14 days in vitro were exposed for 5, 10, or 20 min to either 900 MHz continuous waves or 900 MHz waves modulated in amplitude at 50 Hz using a sinusoidal waveform and 100% modulation index. The strength of the electric field (rms value) at the sample position was 10V/m. No change in cellular viability evaluated by MTT test and lactate dehydrogenase release was observed. A significant increase in ROS levels and DNA fragmentation was found only after exposure of the astrocytes to modulated EMF for 20 min. No evident effects were detected when shorter time intervals or continuous waves were used. The irradiation conditions allowed the exclusion of any possible thermal effect. Our data demonstrate, for the first time, that even acute exposure to low intensity EMF induces ROS production and DNA fragmentation in astrocytes in primary cultures, which also represent the principal target of modulated EMF. Our findings also suggest the hypothesis that the effects could be due to hyperstimulation of the glutamate receptors, which play a crucial role in acute and chronic brain damage. Furthermore, the results show the importance of the amplitude modulation in the interaction between EMF and neocortical astrocytes.

(E) 1 **Cervellati F, Valacchi G, Lunghi L, Fabbri E,** Valbonesi **P, Marci R, Biondi C, Vesce F. 17-β-estradiol counteracts the effects of high frequency electromagnetic fields on trophoblastic connexins and integrins. Oxid Med Cell Longev. 2013;2013:280850. doi: 10.1155/2013/280850. (GE)**

We investigated the effect of high-frequency electromagnetic fields (HF-EMFs) and 17-β-estradiol on connexins (Cxs), integrins (Ints), and estrogen receptor (ER) expression, as well as on ultrastructure of trophoblast-derived HTR-8/SVneo cells. HF-EMF, 17-β-estradiol, and their combination induced an increase of Cx40 and Cx43 mRNA expression. HF-EMF decreased Int alpha1 and β 1 mRNA levels but enhanced Int alpha5 mRNA expression. All the Ints mRNA expressions were increased by 17-β-estradiol and exposure to both stimuli. ER-β mRNA was reduced by HF-EMF but augmented by 17-β-estradiol alone or with HF-EMF. ER-β immunofluorescence showed a cytoplasmic localization in sham and HF-EMF exposed cells which became nuclear after treatment with hormone or both stimuli. Electron microscopy evidenced a loss of cellular contact in exposed cells which appeared counteracted by 17-β-estradiol. We demonstrate that 17-β-estradiol modulates Cxs and Ints as well as ER-β expression induced by HF-EMF, suggesting an influence of both stimuli on trophoblast differentiation and migration.

(NE) Chang SK, Choi JS, Gil HW, Yang JO, Lee EY, Jeon YS, Lee ZW, Lee M, Hong MY, Ho Son T, Hong SY. Genotoxicity evaluation of electromagnetic fields generated by 835-MHz mobile phone frequency band. Eur J Cancer Prev 14:175-179, 2005. (GT, IA) (Some interaction effects with chemicals are reported in this paper.)

It is still unclear whether the exposure to electromagnetic fields (EMFs) generated by mobile phone radiation is directly linked to cancer. We examined the biological effects of an EMF at 835 MHz, the most widely used communication frequency band in Korean CDMA mobile phone networks, on bacterial reverse mutation (Ames assay) and DNA stability (in vitro DNA degradation). In the Ames assay, tester strains alone or combined with positive mutagen were applied in an artificial mobile phone frequency EMF generator with continuous waveform at a specific absorption rate (SAR) of 4 W/kg for 48 h. In the presence of the 835-MHz EMF radiation, incubation with positive mutagen 4-nitroquinoline-1-oxide and cumene hydroxide further increased the mutation rate in Escherichia coli WP2 and TA102, respectively, while the contrary results in Salmonella typhimurium TA98 and TA1535 treated with 4-nitroquinoline-1-oxide and sodium azide, respectively, were shown as antimutagenic. However, these mutagenic or co-mutagenic effects of 835-MHz radiation were not significantly repeated in other relevant strains with same mutation type. In the DNA degradation test, the exposure to 835-MHz EMF did not change the rate of degradation observed using plasmid pBluescriptSK(+) as an indicator. Thus, we suggest that 835-MHz EMF under the conditions of our study neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro.

(NE) Chauhan V, Mariampillai A, Bellier PV, Qutob SS, Gajda GB, Lemay E, Thansandote A, McNamee JP. Gene expression analysis of a human lymphoblastoma cell

line exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Radiat Res. 165(4):424-429, 2006. (GE)

This study was designed to determine whether radiofrequency (RF) fields of the type used for wireless communications could elicit a cellular stress response. As general indicators of a cellular stress response, we monitored changes in proto-oncogene and heat-shock protein expression. Exponentially growing human lymphoblastoma cells (TK6) were exposed to 1.9 GHz pulse-modulated RF fields at average specific absorption rates (SARs) of 1 and 10 W/kg. Perturbations in the expression levels of the proto-oncogenes FOS, JUN and MYC after exposure to sham and RF fields were assessed by real-time RT-PCR. In addition, the transcript levels of the cellular stress proteins HSP27 and inducible HSP70 were also monitored. We demonstrated that transcript levels of these genes in RF-field-exposed cells showed no significant difference in relation to the sham treatment group. However, concurrent positive (heat-shock) control samples displayed a significant elevation in the expression of HSP27, HSP70, FOS and JUN. Conversely, the levels of MYC mRNA were found to decline in the positive (heat-shock) control. In conclusion, our study found no evidence that the 1.9 GHz RF-field exposure caused a general stress response in TK6 cells under our experimental conditions.

(NE) Chauhan V, Mariampillai A, Gajda GB, Thansandote A, McNamee JP. Analysis of proto-oncogene and heat-shock protein gene expression in human derived cell-lines exposed in vitro to an intermittent 1.9 GHz pulse-modulated radiofrequency field. Int J Radiat Biol. 82(5):347-354, 2006. (GE)

Purpose: Several studies have reported that radiofrequency (RF) fields, as emitted by mobile phones, may cause changes in gene expression in cultured human cell-lines. The current study was undertaken to evaluate this possibility in two human-derived immune cell-lines.Materials and methods: HL-60 and Mono-Mac-6 (MM6) cells were individually exposed to intermittent (5 min on, 10 min off) 1.9 GHz pulse-modulated RF fields at a average specific absorption rate (SAR) of 1 and 10 W/kg at 37 +/- 0.5 degrees C for 6 h. Concurrent negative and positive (heat-shock for 1 h at 43 degrees C) controls were conducted with each experiment. Immediately following RF field exposure (T = 6 h) and 18 h post-exposure (T = 24 h), cell pellets were collected from each of the culture dishes and analyzed for transcript levels of proto-oncogenes (c-jun, c-myc and c-fos) and the stress-related genes (heat shock proteins (HSP) HSP27 and HSP70B) by quantitative reverse transcriptase polymerase chain reaction (RT-PCR).Results: No significant effects were observed in mRNA expression of HSP27, HSP70, c-jun, c-myc or c-fos between the sham and RF-exposed groups, in either of the two cell-lines. However, the positive (heat-shock) control group displayed a significant elevation in the expression of HSP27, HSP70, c-fos and c-jun in both cell-lines at T = 6 and 24 h, relative to the sham and negative control groups.Conclusion: This study found no evidence that exposure of cells to non-thermalizing levels of 1.9 GHz pulse-modulated RF fields can cause any detectable change in stress-related gene expression.

(NE) Chauhan V, Qutob SS, Lui S, Mariampillai A, Bellier PV, Yauk CL, Douglas GR, Williams A, McNamee JP. Analysis of gene expression in two human-derived cell lines exposed in vitro to a 1.9 GHz pulse-modulated radiofrequency field. Proteomics. 7(21):3896-3905, 2007. (GE)

There is considerable controversy surrounding the biological effects of radiofrequency (RF) fields, as emitted by mobile phones. Previous work from our laboratory has shown no effect related to the exposure of 1.9 GHz pulse-modulated RF fields on the expression of 22,000 genes in a human glioblastoma-derived cell-line (U87MG) at 6 h following a 4 h RF field exposure period. As a follow-up to this study, we have now examined the effect of RF field exposure on the possible expression of late onset genes in U87MG cells after a 24 h RF exposure period. In addition, a human monocyte-derived cell-line (Mono-Mac-6, MM6) was exposed to intermittent (5 min ON, 10 min OFF) RF fields for 6 h and then gene expression was assessed immediately after exposure and at 18 h postexposure. Both cell lines were exposed to 1.9 GHz pulse-modulated RF fields for 6 or 24 h at specific absorption rates (SARs) of 0.1-10.0 W/kg. In support of our previous results, we found no evidence that nonthermal RF field exposure could alter gene expression in either cultured U87MG or MM6 cells, relative to nonirradiated control groups. However, exposure of both cell-lines to heat-shock conditions (43 degrees C for 1 h) caused an alteration in the expression of a number of well-characterized heat-shock proteins.

(E) Chavdoula ED, Panagopoulos DJ, Margaritis LH. Comparison of biological effects between continuous and intermittent exposure to GSM-900-MHz mobile phone radiation: detection of apoptotic cell-death features. Mutat Res 700:51-61, 2010. (RP, LE, GT)

In the present study we used a 6-min daily exposure of dipteran flies, Drosophila melanogaster, to GSM-900 MHz (Global System for Mobile Telecommunications) mobile phone electromagnetic radiation (EMR), to compare the effects between the continuous and four different intermittent exposures of 6min total duration, and also to test whether intermittent exposure provides any cumulative effects on the insect's reproductive capacity as well as on the induction of apoptotic cell death. According to our previous experiments, a 6-min continuous exposure per day for five days to GSM-900 MHz and DCS-1800 MHz (Digital Cellular System) mobile phone radiation, brought about a large decrease in the insect's reproductive capacity, as defined by the number of F pupae. This decrease was found to be non thermal and correlated with an increased percentage of induced fragmented DNA in the egg chambers' cells at early- and mid-oogenesis. In the present experiments we show that intermittent exposure also decreases the reproductive capacity and alters the actin cytoskeleton network of the egg chambers, another known aspect of cell death that was not investigated in previous experiments, and that the effect is also due to DNA fragmentation. Intermittent exposures with 10-min intervals between exposure sessions proved to be almost equally effective as continuous exposure of the same total duration, whereas longer intervals between the exposures seemed to allow the organism the time required to recover and partly overcome the above-mentioned effects of the GSM exposure.

(E) Chen G, Lu D, Chiang H, Leszczynski D, Xu Z. Using model organism Saccharomyces cerevisiae to evaluate the effects of ELF-MF and RF-EMF exposure on global gene expression. Bioelectromagnetics. 33(7):550-560, 2012 . (GE)

The potential health hazard of exposure to electromagnetic fields (EMF) continues to cause public concern. However, the possibility of biological and health effects of exposure to EMF remains controversial and their biophysical mechanisms are unknown. In the present study, we used Saccharomyces cerevisiae to identify genes responding to extremely low frequency magnetic fields (ELF-MF) and to radiofrequency EMF (RF-EMF) exposures. The yeast cells were exposed for 6 h to either 0.4 mT 50 Hz ELF-MF or 1800 MHz RF-EMF at a specific absorption rate of 4.7 W/kg. Gene expression was analyzed by microarray screening and confirmed using real-time reverse transcription-polymerase chain reaction (RT-PCR). We were unable to confirm microarray-detected changes in three of the ELF-MF responsive candidate genes using RT-PCR ($P > 0.05$). On the other hand, out of the 40 potential RF-EMF responsive genes, only the expressions of structural maintenance of chromosomes 3 (SMC3) and aquaporin 2 (AQY2 (m)) were confirmed, while three other genes, that is, halotolerance protein 9 (HAL9), yet another kinase 1 (YAK1) and one function-unknown gene (open reading frame: YJL171C), showed opposite changes in expression compared to the microarray data ($P < 0.05$). In conclusion, the results of this study suggest that the yeast cells did not alter gene expression in response to 50 Hz ELF-MF and that the response to RF-EMF is limited to only a very small number of genes. The possible biological consequences of the gene expression changes induced by RF-EMF await further investigation. 1

(E) De Iuliis GN, Newey RJ, King BV, Aitken RJ. Mobile phone radiation induces reactive oxygen species production and DNA damage in human spermatozoa in vitro. PLoS One 4:e6446, 2009. (GT, OX, RP)

BACKGROUND: In recent times there has been some controversy over the impact of electromagnetic radiation on human health. The significance of mobile phone radiation on male reproduction is a key element of this debate since several studies have suggested a relationship between mobile phone use and semen quality. The potential mechanisms involved have not been established, however, human spermatozoa are known to be particularly vulnerable to oxidative stress by virtue of the abundant availability of substrates for free radical attack and the lack of cytoplasmic space to accommodate antioxidant enzymes. Moreover, the induction of oxidative stress in these cells not only perturbs their capacity for fertilization but also contributes to sperm DNA damage. The latter has, in turn, been linked with poor fertility, an increased incidence of miscarriage and morbidity in the offspring, including childhood cancer. In light of these associations, we have analyzed the influence of RF-EMR on the cell biology of human spermatozoa in vitro. PRINCIPAL FINDINGS: Purified human spermatozoa were exposed to radio-frequency electromagnetic radiation (RF-EMR) tuned to 1.8 GHz and covering a range of specific absorption rates (SAR) from 0.4 W/kg to 27.5 W/kg. In step with increasing SAR, motility and vitality were significantly reduced after RF-EMR exposure, while the mitochondrial generation of reactive oxygen species and DNA fragmentation were significantly elevated ($P<0.001$). Furthermore, we also observed highly significant relationships between SAR, the oxidative DNA damage bio-marker, 8-OH-dG, and DNA fragmentation after RF-EMR exposure. CONCLUSIONS: RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells while stimulating DNA base adduct formation and, ultimately DNA fragmentation. These findings have clear implications for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring.

(E) Del Vecchio G, Giuliani A, Fernandez M, Mesirca P, Bersani F, Pinto R, Ardoino L, Lovisolo GA, Giardino L, Calzà L. Continuous exposure to 900MHz GSM-modulated EMF alters morphological maturation of neural cells. Neurosci Lett. 455(3):173-177, 2009. (GE, DE)

The effects of radiofrequency electromagnetic field (RF-EMF) exposure on neuronal phenotype maturation have been studied in two different in vitro models: murine SN56 cholinergic cell line and rat primary cortical neurons. The samples were exposed at a dose of 1W/kg at 900 MHz GSM modulated. The phenotype analysis was carried out at 48 and 72 h (24 and 48 h of SN56 cell line differentiation) or at 24, 72, 120 h (2, 4 and 6 days in vitro for cortical neurons) of exposure, on live and immunolabeled neurons, and included the morphological study of neurite emission, outgrowth and branching. Moreover, cortical neurons were studied to detect alterations in the expression pattern of cytoskeleton regulating factors, e.g. beta-thymosin, and of early genes, e.g. c-Fos and c-Jun through real-time PCR on mRNA extracted after 24h exposure to EMF. We found that RF-EMF exposure reduced the number of neurites generated by both cell systems, and this alteration correlates to increased expression of beta-thymosin mRNA.

(E) Deshmukh PS, Megha K, Banerjee BD, Ahmed RS, Chandna S, Abegaonkar MP, Tripathi AK. Detection of Low Level Microwave Radiation Induced Deoxyribonucleic Acid Damage Vis-à-vis Genotoxicity in Brain of Fischer Rats. Toxicol Int. 20(1):19-24, 2013. (GT, LE)

BACKGROUND: Non-ionizing radiofrequency radiation has been increasingly used in industry, commerce, medicine and especially in mobile phone technology and has become a matter of serious concern in present time. OBJECTIVE: The present study was designed to investigate the possible deoxyribonucleic acid (DNA) damaging effects of low-level microwave radiation in brain of Fischer rats. MATERIALS AND METHODS: Experiments were performed on male Fischer rats exposed to microwave radiation for 30 days at three different frequencies: 900, 1800 and 2450 MHz. Animals were divided into 4 groups: Group I (Sham exposed): Animals not exposed to microwave radiation but kept under same conditions as that of other groups, Group II: Animals exposed to microwave radiation at frequency 900 MHz at specific absorption rate (SAR) 5.953 × 10(-4) W/kg, Group III: Animals exposed to 1800 MHz at SAR 5.835 × 10(-4) W/kg and Group IV: Animals exposed to 2450 MHz at SAR 6.672 × 10(-4) W/kg. At the end of the exposure period animals were sacrificed immediately and DNA damage in brain tissue was assessed using alkaline comet assay. RESULTS: In the present study, we demonstrated DNA damaging effects of low level microwave radiation in brain. CONCLUSION: We concluded that low SAR microwave radiation exposure at these frequencies may induce DNA strand breaks in brain tissue.

(E) Engelmann JC, Deeken R, Müller T, Nimtz G, Roelfsema MR, Hedrich R. Is gene activity in plant cells affected by UMTS-irradiation? A whole genome approach. Adv Appl Bioinform Chem. 1:71-83, 2008. (GE)

Mobile phone technology makes use of radio frequency (RF) electromagnetic fields transmitted through a dense network of base stations in Europe. Possible harmful effects of RF fields on humans and animals are discussed, but their effect on plants has received little attention. In search for physiological processes of plant cells sensitive to RF fields, cell suspension cultures of Arabidopsis thaliana were exposed for 24 h to a RF field protocol representing typical microwave exposition in an urban environment. mRNA of exposed cultures and controls was used to hybridize Affymetrix-ATH1 whole genome microarrays. Differential expression analysis revealed significant changes in transcription of 10 genes, but they did not exceed a fold change of 2.5. Besides that 3 of them are dark-inducible, their functions do not point to any known responses of plants to environmental stimuli. The changes in transcription of these genes were compared with published microarray datasets and revealed a weak similarity of the microwave to light treatment experiments. Considering the large changes described in published experiments, it is questionable if the small alterations caused by a 24 h continuous microwave exposure would have any impact on the growth and reproduction of whole plants.

(E) Esmekaya MA, Aytekin E, Ozgur E, Güler G, Ergun MA, Omeroğlu S, Seyhan N. Mutagenic and morphologic impacts of 1.8GHz radiofrequency radiation on human peripheral blood lymphocytes (hPBLs) and possible protective role of pre-treatment with Ginkgo biloba (EGb 761). Sci Total Environ. 410-411:59-64, 2011. (GT, OX)

The mutagenic and morphologic effects of 1.8GHz Global System for Mobile Communications (GSM) modulated RF (radiofrequency) radiation alone and in combination with Ginkgo biloba (EGb 761) pre-treatment in human peripheral blood lymphocytes (hPBLs) were investigated in this study using Sister Chromatid Exchange (SCE) and electron microscopy. Cell viability was assessed with 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) reduction assay. The lymphocyte cultures were exposed to GSM modulated RF radiation at 1.8GHz for 6, 8, 24 and 48h with and without EGb 761. We observed morphological changes in pulse-modulated RF radiated lymphocytes. Longer exposure periods led to destruction of organelle and nucleus structures. Chromatin change and the loss of mitochondrial crista occurred in cells exposed to RF for 8h and 24h and were more pronounced in cells exposed for 48h. Cytoplasmic lysis and destruction of membrane integrity of cells and nuclei were also seen in 48h RF exposed cells. There was a significant increase ($p<0.05$) in SCE frequency in RF exposed lymphocytes compared to sham controls. EGb 761 pre-treatment significantly decreased SCE from RF radiation. RF radiation also inhibited cell viability in a time dependent manner. The inhibitory effects of RF radiation on the growth of lymphoctes were marked in longer exposure periods. EGb 761 pre-treatment significantly increased cell viability in RF+EGb 761 treated groups at 8 and 24h when compared to RF exposed groups alone. The results of our study showed that RF radiation affects cell morphology, increases SCE and inhibits cell proliferation. However, EGb 761 has a protective role against RF induced mutagenity. We concluded that RF radiation induces chromosomal damage in hPBLs but this damage may be reduced by EGb 761 pre-treatment.

(NE) Falzone N, Huyser C, Franken DR, Leszczynski D. Mobile phone radiation does not induce pro-apoptosis effects in human spermatozoa. Radiat Res 174:169-176, 2010. (GT, OX)

Abstract Recent reports suggest that mobile phone radiation may diminish male fertility. However, the effects of this radiation on human spermatozoa are largely unknown. The present study examined effects of the radiation on induction of apoptosis-related properties in human spermatozoa. Ejaculated, density-purified, highly motile human spermatozoa were exposed to mobile phone radiation at specific absorption rates (SARs) of 2.0 and 5.7 W/kg. At various times after exposure, flow cytometry was used to examine caspase 3 activity, externalization of phosphatidylserine (PS), induction of DNA strand breaks, and generation of reactive oxygen species. Mobile phone radiation had no statistically significant effect on any of the parameters studied. This suggests that the impairment of fertility reported in some studies was not caused by the induction of apoptosis in spermatozoa.

(E) Ferreira AR, Knakievicz T, de Bittencourt Pasquali MA, Gelain DP, Dal-Pizzol F, Fernandez CE, de Almeida de Salles AA, Ferreira HB, Moreira JC. Ultra high frequency-electromagnetic field irradiation during pregnancy leads to an increase in erythrocytes micronuclei incidence in rat offspring. Life Sci 80: 43-50, 2006. (GT, OX, LE, DE)

Mobile telephones and their base stations are an important ultra high frequency-electromagnetic field (UHF-EMF) source and their utilization is increasing all over the world. Epidemiological studies suggested that low energy UHF-EMF emitted from a cellular telephone may cause biological effects, such as DNA damage and changes on oxidative metabolism. An in vivo mammalian cytogenetic test, the micronucleus (MN) assay, was used to investigate the occurrence of chromosomal damage in erythrocytes from rat offspring exposed to a non-thermal UHF-EMF from a cellular phone during their embryogenesis; the irradiated group showed a significant increase in MN occurrence. In order to investigate if UHF-EMF could also alter oxidative parameters in the peripheral blood and in the liver - an important hematopoietic tissue in rat embryos and newborns - we also measured the activity of antioxidant enzymes, quantified total sulfhydryl content, protein carbonyl groups, thiobarbituric acid-reactive species and total non-enzymatic antioxidant defense. No significant differences were found in any oxidative parameter of offspring blood and liver. The average number of pups in each litter has also not been significantly altered. Our results suggest that, under our experimental conditions, UHF-EMF is able to induce a genotoxic response in hematopoietic tissue during the embryogenesis through an unknown mechanism.

(NE) Finnie JW, Cai Z, Blumbergs PC, Manavis J, Kuchel TR. Expression of the immediate early gene, c-fos, in fetal brain after whole of gestation exposure of pregnant mice to global system for mobile communication microwaves. Pathology. 38(4):333-335, 2006. (GE, DE)

AIMS: To study immediate early gene, c-fos, expression as a marker of neural stress after whole of gestation exposure of the fetal mouse brain to mobile telephone-type radiofrequency fields. METHODS: Using a purpose-designed exposure system at 900 MHz, pregnant mice were given a single, far-field, whole body exposure at a specific absorption rate of 4 W/kg for 60 min/day from day 1 to day 19 of gestation. Pregnant control mice were sham-exposed or freely mobile in a cage without further restraint. Immediately prior to parturition on gestational day 19, fetal heads were collected, fixed in 4% paraformaldehyde and paraffin embedded. Any stress response in the brain was detected by c-fos immunohistochemistry in the cerebral cortex, basal ganglia, thalamus, hippocampus, midbrain, cerebellum and medulla. RESULTS: c-fos expression was of limited, but consistent, neuroanatomical distribution and there was no difference in immunoreactivity between exposed and control brains. CONCLUSION: In this animal model, no stress response was detected in the fetal brain using c-fos immunohistochemistry after whole of gestation exposure to mobile telephony.

(E) Franzellitti S, Valbonesi P, Ciancaglini N, Biondi C, Contin A, Bersani F, Fabbri E. Transient DNA damage induced by high-frequency electromagnetic fields (GSM 1.8 GHz)

in the human trophoblast HTR-8/SVneo cell line evaluated with the alkaline comet assay. Mutat Res 683(1-2):35-42, 2010. (GT, WS)

One of the most controversial issue regarding high-frequency electromagnetic fields (HF-EMF) is their putative capacity to affect DNA integrity. This is of particular concern due to the increasing use of HF-EMF in communication technologies, including mobile phones. Although epidemiological studies report no detrimental effects on human health, the possible disturbance generated by HF-EMF on cell physiology remains controversial. In addition, the question remains as to whether cells are able to compensate their potential effects. We have previously reported that a 1-h exposure to amplitude-modulated 1.8 GHz sinusoidal waves (GSM-217 Hz, SAR=2 W/kg) largely used in mobile telephony did not cause increased levels of primary DNA damage in human trophoblast HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations were considered of interest. In the present work, HTR-8/SVneo cells were exposed for 4, 16 or 24h to 1.8 GHz continuous wave (CW) and different GSM signals, namely GSM-217 Hz and GSM-Talk (intermittent exposure: 5 min field on, 10 min field off). The alkaline comet assay was used to evaluate primary DNA damages and/or strand breaks due to uncompleted repair processes in HF-EMF exposed samples. The amplitude-modulated signals GSM-217 Hz and GSM-Talk induced a significant increase in comet parameters in trophoblast cells after 16 and 24h of exposure, while the un-modulated CW was ineffective. However, alterations were rapidly recovered and the DNA integrity of HF-EMF exposed cells was similar to that of sham-exposed cells within 2h of recovery in the absence irradiation. Our data suggest that HF-EMF with a carrier frequency and modulation scheme typical of the GSM signal may affect the DNA integrity.

(E) Furtado-Filho OV, Borba JB, Dallegrave A, Pizzolato TM, Henriques JA, Moreira JC, Saffi J. Effect of 950 MHz UHF electromagnetic radiation on biomarkers of oxidative damage, metabolism of UFA and antioxidants in the livers of young rats of different ages. Int J Radiat Biol. 2013 Jul 25. [Epub ahead of print] (LE, GT, OX)

Purpose: To assess the effect of 950 MHz ultra-high-frequency electromagnetic radiation (UHF EMR) on biomarkers of oxidative damage, as well as to verify the concentration of unsaturated fatty acids (UFA) and the expression of the catalase in the livers of rats of different ages. Materials and methods: Twelve rats were equally divided into two groups as controls (CR) and exposed (ER), for each age (0, 6, 15 and 30 days). Radiation exposure lasted half an hour per day for up to 51 days (21 days of gestation and 6, 15 or 30 days of life outside the womb). The specific absorption rate (SAR) ranged from 1.3-1.0 W/kg. The damage to lipids, proteins and DNA was verified by thiobarbituric acid reactive substances (TBARS), protein carbonyls and comets, respectively. UFA were determined by gas chromatography with a flame ionization detector. The expression of catalase was by Western blotting. Results: The neonates had low levels of TBARS and concentrations of UFA after exposure. There was no age difference in the accumulation of protein carbonyls for any age. The DNA damage of ER 15 or 30 days was different. The exposed neonates exhibited lower expression of catalase. Conclusions: 950 MHz UHF EMR does not cause oxidative stress (OS), and it is not genotoxic to the livers of neonates or those of 6 and 15 day old rats, but it changes the concentrations of polyunsaturated fatty acid (PUFA) in neonates. For rats of 30 days, no OS, but it is genotoxic to the livers of ER to total body irradiation.

(E) Gajski G, Garaj-Vrhovac V. Radioprotective effects of honeybee venom (Apismellifera) against 915-MHz microwave radiation-induced DNA damage in wistar rat lymphocytes: in vitro study. Int J Toxicol 28:88-98, 2009. (GT, OX)

The aim of this study is to investigate the radioprotective effect of bee venom against DNA damage induced by 915-MHz microwave radiation (specific absorption rate of 0.6 W/kg) in Wistar rats. Whole blood lymphocytes of Wistar rats are treated with 1 microg/mL bee venom 4 hours prior to and immediately before irradiation. Standard and formamidopyrimidine-DNA glycosylase (Fpg)-modified comet assays are used to assess basal and oxidative DNA damage produced by reactive oxygen species. Bee venom shows a decrease in DNA damage compared with irradiated samples. Parameters of Fpg-modified comet assay are statistically different from controls, making this assay more sensitive and suggesting that oxidative stress is a possible mechanism of DNA damage induction. Bee venom is demonstrated to have a radioprotective effect against basal and oxidative DNA damage. Furthermore, bee venom is not genotoxic and does not produce oxidative damage in the low concentrations used in this study.

(E) Gandhi G, Anita, Genetic damage in mobile phone users: some preliminary findings. Ind J Hum Genet 11:99-104, 2005. (GT, HU)

BACKGROUND: The impact of microwave (MW)/radio frequency radiation (RFR) on important biological parameters is probably more than a simply thermal one. Exposure to radio frequency (RF) signals generated by the use of cellular telephones have increased dramatically and reported to affect physiological, neurological, cognitive and behavioural changes and to induce, initiate and promote carcinogenesis. Genotoxicity of RFR has also been reported in various test systems after in vitro and/or in vivo exposure but none in mobile phone users. AIMS: In the present study, DNA and chromosomal damage investigations were carried out on the peripheral blood lymphocytes of individuals using mobile phones, being exposed to MW frequency ranging from 800 to 2000 MHz. METHODS: DNA damage was assessed using the single cell gel electrophoresis assay and aneugenic and clastogenic damage by the in vivo capillary blood micronucleus test (MNT) in a total of 24 mobile phone users. RESULTS: Mean comet tail length (26.76 ± 0.054 mm; 39.75% of cells damaged) in mobile phone users was highly significant from that in the control group. The in vivo capillary blood MNT also revealed highly significant (0.25) frequency of micronucleated (MNd) cells. CONCLUSIONS: These results highlight a correlation between mobile phone use (exposure to RFR) and genetic damage and require interim public health actions in the wake of widespread use of mobile telephony.

(E) Gandhi G, Singh P. Cytogenetic damage in mobile phone users: preliminary data. Int J Hum Genet 5:259-265, 2005. (GT, HU)

Mobile telephones, sometimes called cellular (cell) phones or handies, are now an integral part of modern life. The mobile phone handsets are low-powered radiofrequency transmitters, emitting maximum powers in the range of 0.2 to 0.6 watts. Scientific concenrns have increased sufficiently over the possible hazard to health from using cell phones. The reported adverse health effects include physiological, behavioural and cognitive changes as well as tumour formation and genetic damage. However findings are controversial and no consensus exists. Genotoxicity has been observed either in lower organisms or in vitro studies. The aim of the present study hence was to detect any cytogenertic damage in mobile phone users by analysing short term peripheral lymphocyte cultures for chromosomal aberrations and the buccal mucosal cells for micronuclei (aneugenicity and clastogenicity). The results revealed increased number of micronucleated buccal cells and cytological abnormalities in cultured lymphocytes indicating the genotoxic response from mobile phone use.

(E) Garaj-Vrhovac V, Gajski G, Pažanin S, Sarolić A, Domijan AM, Flajs D, Peraica M. Assessment of cytogenetic damage and oxidative stress in personnel occupationally exposed to the pulsed microwave radiation of marine radar equipment. Int J Hyg Environ Health. 4(1):59-65, 2011. (GT, HU, OX)

Due to increased usage of microwave radiation, there are concerns of its adverse effect in today's society. Keeping this in view, study was aimed at workers occupationally exposed to pulsed microwave radiation, originating from marine radars. Electromagnetic field strength was measured at assigned marine radar frequencies (3 GHz, 5.5 GHz and 9.4 GHz) and corresponding specific absorption rate values were determined. Parameters of the comet assay and micronucleus test were studied both in the exposed workers and in corresponding unexposed subjects. Differences between mean tail intensity (0.67 vs. 1.22) and moment (0.08 vs. 0.16) as comet assay parameters and micronucleus test parameters (micronuclei, nucleoplasmic bridges and nuclear buds) were statistically significant between the two examined groups, suggesting that cytogenetic alterations occurred after microwave exposure. Concentrations of glutathione and malondialdehyde were measured spectrophotometrically and using high performance liquid chromatography. The glutathione concentration in exposed group was significantly lower than in controls (1.24 vs. 0.53) whereas the concentration of malondialdehyde was significantly higher (1.74 vs. 3.17), indicating oxidative stress. Results suggests that pulsed microwaves from working environment can be the cause of genetic and cell alterations and that oxidative stress can be one of the possible mechanisms of DNA and cell damage.

(E) Guler G, Tomruk A, Ozgur E, Seyhan N.The effect of radiofrequency radiation on DNA and lipid damage in non-pregnant and pregnant rabbits and their newborns. Gen Physiol Biophys 29:59-66, 2010. (GT, OX, LE, DE)

The concerns of people on possible adverse health effects of radiofrequency radiation (RFR) generated from mobile phones as well as their supporting transmitters (base stations) have increased markedly. RFR effect on oversensitive people, such as pregnant women and their developing fetuses, and older people is another source of concern that should be considered. In this study, oxidative DNA damage and lipid peroxidation levels in the brain tissue of pregnant and non-pregnant New Zealand White rabbits and their newborns exposed to RFR were investigated. Thirteen-month-old rabbits were studied in four groups as non-pregnant-control, non-pregnant-RFR exposed, pregnant-control and pregnant-RFR exposed. They were exposed to RFR (1800 MHz GSM; 14 V/m as reference level) for 15 min/day during 7 days. Malondialdehyde (MDA) and 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels were analyzed. MDA and 8-OHdG levels of non-pregnant and pregnant-RFR exposed animals significantly increased with respect to controls ($p < 0.001$, Mann-Whitney test). No difference was found in the newborns ($p > 0.05$, Mann-Whitney). There exist very few experimental studies on the effects of RFR during pregnancy. It would be beneficial to increase the number of these studies in order to establish international standards for the protection of pregnant women from RFR.

(E) Güler G, Tomruk A, Ozgur E, Sahin D, Sepici A, Altan N, Seyhan N. The effect of radiofrequency radiation on DNA and lipid damage in female and male infant rabbits. Int J Radiat Biol. 88(4):367-373, 2012. (LE, GT, OX, DE)

PURPOSE: We aimed to design a prolonged radiofrequency (RF) radiation exposure and investigate in an animal model, possible bio-effects of RF radiation on the ongoing developmental stages of children from conception to childhood. **MATERIALS AND METHODS:** A total of 72 New Zealand female and male white rabbits aged one month were used. Females were exposed to RF radiation for 15 min/day during 7 days, whereas males were exposed to the same level of radiation for 15 min/day during 14 days. Thirty-six female and 36 male infant rabbits were randomly divided into four groups: Group I [Intrauterine (IU) exposure (-); Extrauterine (EU) exposure (-)]: Sham exposure which means rabbits were exposed to 1800 MHz Global System for Mobile Telecommunication (GSM)-like RF signals neither in the IU nor in the EU periods. Group II [IU exposure (-); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals when they reached one month of age. Group III [IU exposure (+); EU exposure (-)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals in the IU period (between 15th and 22nd days of the gestational period). Group IV [IU exposure (+); EU exposure (+)]: Infant rabbits were exposed to 1800 MHz GSM-like RF signals both in the IU period (between 15th and 22nd days of the gestational period) and in the EU period when they reached one month of age. Biochemical analysis for lipid peroxidation and DNA damage were carried out in the livers of all rabbits. **RESULTS:** Lipid peroxidation levels in the liver tissues of female and male infant rabbits increased under RF radiation exposure. Liver 8-hydroxy-2'-deoxyguanosine (8-OHdG) levels of female rabbits exposed to RF radiation were also found to increase when compared with the levels of non-exposed infants. However, there were no changes in liver 8-OHdG levels of male rabbits under RF exposure. **CONCLUSION:** Consequently, it can be concluded that GSM-like RF radiation may induce biochemical changes by increasing free radical attacks to structural biomolecules in the rabbit as an experimental animal model.

(NE) Gurbuz N, Sirav B, Yuvaci HU, Turhan N, Coskun ZK, Seyhan N. Is there any possible genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 MHz GSM-like modulated radio frequency radiation (RFR)? Electromagn Biol Med. 29(3):98-104, 2010. (LE, GT)

People are exposed to many carcinogenic and mutagenic chemicals in their everyday lives. These include antineoplastic drugs, Polycyclic aromatic hydrocarbons (PAH)s, aromatic amines, nitrosamines, metals, and electromagnetic radiation. Based on the state of knowledge acquired during the last 50 years of research on possible biological effects of electromagnetic fields (EMF), the majority of the scientific community is convinced that exposure to EMF below the existing security limits does not cause a risk to the health of the general public. However, this position is questioned by others, who are of the opinion that the available research data are contradictory or inconsistent and, therefore, unreliable. In this study, we aimed to investigate if there is any effect of 1800 MHz GSM modulated radio frequency radiation (RFR) on the number of micronucleus in exfoliated bladder cells of rat which will be informative about the genotoxic damage. Exposure period was 20 min/day, 5 days/week during a month. Six female Wistar rats were used for two groups: Group I (n=6): controls; Group II (n=6): 1.8 GHz exposed animals.

1800 MHz RFR did not showed a significant MN frequencies in rat bladder cells when compared with the control group (p>0.05). 1800 MHz RFR-exposed animals did not produce any genotoxic effect when compared with the control group (p>0.05). Kinetic studies are important for any biomarker, especially those in which tissue differentiation and maturation processes will heavily influence the time between induction of damage and collection of damaged cells for micronucleus analysis.

(NE) **Gurbuz N, Sirav B, Colbay M, Yetkin I, Seyhan N. No genotoxic effect in exfoliated bladder cells of rat under the exposure of 1800 and 2100-MHz radio frequency radiation. Electromagn Biol Med. 2013 Nov 27. [Epub ahead of print]** **(GT, LE)**

Abstract In this study, we aimed to investigate the effects of 1800 and 2100 MHz Radio Frequency (RF) radiation on the number of micronucleus (MN) in exfoliated bladder cells of rat which shows the genotoxic damage. Exposure period was 30 min/day, 6 days/week for a month and two months exposure periods. Thirty male wistar albino rats were used for five groups: Group I (n = 6): 1800 MHz RF exposed animals for one month, Group II (n = 6): 2100 MHz RF exposed animals for one month, Group III (n = 6): 2100 MHz RF exposed for two months, Group IV (n = 6): control group for one month, Group V (n = 6): control group for two months. Rats of the control groups were housed in their home cages during the entire experimental period without subjecting to any experimental manipulation. 1800 and 2100 MHz RF exposures did not result in any significant MN frequencies in rat bladder cells with respect to the control groups (p > 0.05). There was no statistically significant difference between 2100 MHz RF exposed groups, either. Further studies are needed to demonstrate if there is any genotoxic effect, micronucleus formation in other tissues of rats.

(NE) **Hansteen IL, Lågeide L, Clausen KO, Haugan V, Svendsen M, Eriksen JG, Skiaker R, Hauger E, Vistnes AI, Kure EH. Cytogenetic effects of 18.0 and 16.5 GHz microwave radiation on human lymphocytes in vitro. Anticancer Res 29:2885-2892, 2009.** **(GT, IA, WS)**

BACKGROUND: There are few cell studies on the direct genotoxic effects of microwave radiation. In this study, cytogenetic effects of microwave radiation alone or in combination with mitomycin C (MMC) were investigated. MATERIALS AND METHODS: Lymphocytes from two smoking and four non-smoking donors were exposed for 53 hours in vitro to 1.0 W/m continuous-wave radiation at 18.0 GHz or 10 W/m pulsed-wave at 16.5 GHz, alone or in combination with MMC. DNA synthesis and repair were inhibited in vitro in some cultures. RESULTS: No synergistic effect was observed in cells exposed to combinations of microwave radiation and in vitro exposure to MMC, or to cells pre-exposed in vivo to tobacco smoke. For the 16.5 GHz pulsed exposure, a non-significant trend consisting of an increase in aberration frequencies with microwave radiation was shown for the DNA synthesis and repair inhibited cultures both with and without MMC. CONCLUSION: Neither 18.0 GHz continuous-wave nor 16.5 GHz pulsed-wave exposure to human lymphocytes in vitro induced statistically significant increases in chromosomal aberration frequencies. 16.5 GHz pulsed-wave exposure requires further documentation before a true negative conclusion can be drawn.

(NE) **Hansteen IL, Clausen KO, Haugan V, Svendsen M, Svendsen MV, Eriksen JG, Skiaker R, Hauger E, Lågeide L, Vistnes AI, Kure EH. Cytogenetic effects of exposure to**

2.3 GHz radiofrequency radiation on human lymphocytes in vitro. Anticancer Res 29:4323-4330, 2009. (GT, IA)

BACKGROUND: No previous in vitro studies have tested radio frequency radiation for at least one full cell cycle in culture. The aim was to test if exposure used in mobile phones and wireless network technologies would induce DNA damage in cultured human lymphocytes with and without a known clastogen. MATERIALS AND METHODS: Lymphocytes from six donors were exposed to 2.3 GHz, 10 W/m continuous waves, or 2.3 GHz, 10 W/m pulsed waves (200 Hz pulse frequency, 50% duty cycle). Mitomycin C was added to half of the cultures. DNA synthesis and repair were inhibited in one experiment. RESULTS: No statistically significant differences were observed between control and exposed cultures. A weak trend for more chromosomal damage with the interaction of pulsed fields with mitomycin C compared to a constant field was observed. CONCLUSION: Exposure during the whole cell cycle in inhibited cultures did not resulted in significant differences in chromosomal aberrations as compared to controls.

(E) Hekmat A, Saboury AA, Moosavi-Movahedi AA. The toxic effects of mobile phone radiofrequency (940MHz) on the structure of calf thymus DNA. Ecotoxicol Environ Saf. 2012 Nov 16. pii: S0147-6513(12)00368-5. doi: 10.1016/j.ecoenv.2012.10.016. [Epub ahead of print] (GT)

Currently, the biological effects of nonionizing electromagnetic fields (EMFs) including radiofrequency (RF) radiation have been the subject of numerous experimental and theoretical studies. The aim of this study is to evaluate the possible biological effects of mobile phone RF (940MHz, 15V/m and SAR=40mW/kg) on the structure of calf thymus DNA (ct DNA) immediately after exposure and 2h after 45min exposure via diverse range of spectroscopic instruments. The UV-vis and circular dichroism (CD) experiments depict that mobile phone EMFs can remarkably cause disturbance on ct DNA structure. In addition, the DNA samples, immediately after exposure and 2h after 45min exposure, are relatively thermally unstable compared to the DNA solution, which was placed in a small shielded box (unexposed ct DNA). Furthermore, the exposed DNA samples (the DNA samples that were exposed to 940MHz EMF) have more fluorescence emission when compared with the unexposed DNA, which may have occurred attributable to expansion of the exposed DNA structure. The results of dynamic light scattering (DLS) and zeta potential experiments demonstrate that RF-EMFs lead to increment in the surface charge and size of DNA. The structure of DNA immediately after exposure is not significantly different from the DNA sample 2h after 45min exposure. In other words, the EMF-induced conformational changes are irreversible. Collectively, our results reveal that 940MHz can alter the structure of DNA. The displacement of electrons in DNA by EMFs may lead to conformational changes of DNA and DNA disaggregation. Results from this study could have an important implication on the health effects of RF-EMFs exposure. In addition, this finding could proffer a novel strategy for the development of next generation of mobile phone.

(NE) Hintzsche H, Stopper H. Micronucleus frequency in buccal mucosa cells of mobile phone users. Toxicol Lett. 193(1):124-130, 2010. (GT, HU)

Mobile phones are being used extensively throughout the world, with more than four billion accounts existing in 2009. This technology applies electromagnetic radiation in the microwave range. Health effects of this radiation have been subject of debate for a long time, both within the scientific community and within the general public. This study investigated the effect of mobile phone use on genomic instability of the human oral cavity's mucosa cells. 131 Individuals donated buccal mucosa cells extracted by slightly scraping the oral cavity with a cotton swab. Every participant filled out a questionnaire about mobile phone use including duration of weekly use, overall period of exposure and headset usage. 13 Individuals did not use mobile phones at all, 85 reported using the mobile phone for three hours per week or less, and 33 reported use of more than three hours per week. Additionally, information on age, gender, body weight, smoking status, medication and nutrition was retrieved. For staining of the cells a procedure using alpha-tubulin-antibody and chromomycin A(3) was applied. Micronuclei and other markers were evaluated in 1000 cells per individual at the microscope. A second scorer counted another 1000 cells, resulting in 2000 analyzed cells per individual. Mobile phone use did not lead to a significantly increased frequency of micronuclei.

(NE) Hintzsche H, Jastrow C, Kleine-Ostmann T, Schrader T, Stopper H. 900 MHz radiation does not induce micronucleus formation in different cell types. Mutagenesis. 27(4):477-483, 2012 . (GT)

The exposure of the population to non-ionising electromagnetic radiation is still increasing, mainly due to mobile communication. Whether low-intensity electromagnetic fields can cause other effects apart from heating has been a subject of debate. One of the effects, which were proposed to be caused by mobile phone radiation, is the occurrence of mitotic disturbances. The aim of this study was to investigate possible consequences of these mitotic disturbances as manifest genomic damage, i.e. micronucleus induction. Cells were irradiated at a frequency of 900 MHz, which is located in one of the main frequency bands applied for mobile communication. Two cell types were used, HaCaT cells as human cells and A(L) cells (human-hamster hybrid cells), in which mitotic disturbances had been reported to occur. After different post-exposure incubation periods, cells were fixed and micronucleus frequencies were evaluated. Both cell types did not show any genomic damage after exposure. To adapt the protocol for the micronucleus test into the direction of the protocol for mitotic disturbances, the post-exposure incubation period was reduced and exposure time was extended to one cell cycle length. This did not result in any increase of the genomic damage. In conclusion, micronucleus induction was not observed as a consequence of exposure to non-ionising radiation, even though this agent was reported to cause mitotic disturbances under similar experimental conditions.

(NE) Hirose H, Sakuma N, Kaji N, Suhara T, Sekijima M, Nojima T, Miyakoshi J. Phosphorylation and gene expression of p53 are not affected in human cells exposed to 2.1425 GHz band CW or W-CDMA modulated radiation allocated to mobile radio base stations. Bioelectromagnetics 27:494-504, 2006. (GT)

A large-scale in vitro study focusing on low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields induce apoptosis or other cellular stress response that activate p53 or the p53-signaling pathway. First, we evaluated the response of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction by the International Commission on Non-Ionizing

Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and wideband code division multiple access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced apoptosis or any signs of stress. Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg, and CW radiation at 80 mW/kg for 24 or 48 h. Human IMR-90 fibroblasts from fetal lungs were exposed to both W-CDMA and CW radiation at a SAR of 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the percentage of apoptotic cells were observed between the test groups exposed to RF signals and the sham-exposed negative controls, as evaluated by the Annexin V affinity assay. No significant differences in expression levels of phosphorylated p53 at serine 15 or total p53 were observed between the test groups and the negative controls by the bead-based multiplex assay. Moreover, microarray hybridization and real-time RT-PCR analysis showed no noticeable differences in gene expression of the subsequent downstream targets of p53 signaling involved in apoptosis between the test groups and the negative controls. Our results confirm that exposure to low-level RF signals up to 800 mW/kg does not induce p53-dependent apoptosis, DNA damage, or other stress response in human cells.

(NE) Hirose H, Sakuma N, Kaji N, Nakayama K, Inoue K, Sekijima M, Nojima T, Miyakoshi J. Mobile phone base station-emitted radiation does not induce phosphorylation of Hsp27. Bioelectromagnetics 28:99-108, 2007. (GE)

An in vitro study focusing on the effects of low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields act to induce phosphorylation and overexpression of heat shock protein hsp27. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced activation or gene expression of hsp27 and other heat shock proteins (hsps). Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80 and 800 mW/kg for 2-48 h, and CW radiation at 80 mW/kg for 24 h. Human IMR-90 fibroblasts from fetal lungs were exposed to W-CDMA at 80 and 800 mW/kg for 2 or 28 h, and CW at 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the expression levels of phosphorylated hsp27 at serine 82 (hsp27[pS82]) were observed between the test groups exposed to W-CDMA or CW signal and the sham-exposed negative controls, as evaluated immediately after the exposure periods by bead-based multiplex assays. Moreover, no noticeable differences in the gene expression of hsps were observed between the test groups and the negative controls by DNA Chip analysis. Our results confirm that exposure to low-level RF field up to 800 mW/kg does not induce phosphorylation of hsp27 or expression of hsp gene family.

(NE) Huang TQ, Lee MS, Oh E, Zhang BT, Seo JS, Park WY. Molecular responses of Jurkat T-cells to 1763 MHz radiofrequency radiation.Int J RadiatBiol 84:734-741, 2008. (GT, GE)

PURPOSE: The biological effects of exposure to mobile phone emitted radiofrequency (RF) radiation are the subject of intense study, yet the hypothesis that RF exposure is a potential health hazard remains controversial. In this paper, we monitored cellular and molecular changes in Jurkat human T lymphoma cells after irradiating with 1763 MHz RF radiation to understand the effect on RF radiation in immune cells. MATERIALS AND METHODS: Jurkat T-cells were exposed to RF radiation to assess the effects on cell proliferation, cell cycle progression, DNA damage and gene expression. Jurkat cells were exposed to 1763 MHz RF radiation at 10 W/kg specific absorption rate (SAR) and compared to sham exposed cells. RESULTS: RF exposure did not produce significant changes in cell numbers, cell cycle distributions, or levels of DNA damage. In genome-wide analysis of gene expressions, there were no genes changed more than two-fold upon RF-radiation while ten genes change to 1.3 approximately 1.8-fold. Among ten genes, two cytokine receptor genes such as chemokine (C-X-C motif) receptor 3 (CXCR3) and interleukin 1 receptor, type II (IL1R2) were down-regulated upon RF radiation, but they were not directly related to cell proliferation or DNA damage responses. CONCLUSION: These results indicate that the alterations in cell proliferation, cell cycle progression, DNA integrity or global gene expression was not detected upon 1763 MHz RF radiation under 10 W/kg SAR for 24 h to Jurkat T cells.

(NE) Huang TQ, Lee MS, Oh EH, Kalinec F, Zhang BT, Seo JS, Park WY. Characterization of biological effect of 1763 MHz radiofrequency exposure on auditory hair cells.Int J Radiat Biol 84:909-915, 2008. (GT, GE)

Purpose: Radiofrequency (RF) exposure at the frequency of mobile phones has been reported not to induce cellular damage in in vitro and in vivo models. We chose HEI-OC1 immortalized mouse auditory hair cells to characterize the cellular response to 1763 MHz RF exposure, because auditory cells could be exposed to mobile phone frequencies. Materials and methods: Cells were exposed to 1763 MHz RF at a 20 W/kg specific absorption rate (SAR) in a code division multiple access (CDMA) exposure chamber for 24 and 48 h to check for changes in cell cycle, DNA damage, stress response, and gene expression. Results: Neither of cell cycle changes nor DNA damage was detected in RF-exposed cells. The expression of heat shock proteins (HSP) and the phosphorylation of mitogen-activated protein kinases (MAPK) did not change, either. We tried to identify any alteration in gene expression using microarrays. Using the Applied Biosystems 1700 full genome expression mouse microarray, we found that only 29 genes (0.09% of total genes examined) were changed by more than 1.5-fold on RF exposure. Conclusion: From these results, we could not find any evidence of the induction of cellular responses, including cell cycle distribution, DNA damage, stress response and gene expression, after 1763 MHz RF exposure at an SAR of 20 W/kg in HEI-OC1 auditory hair cells.

(E) Jiang B, Nie J, Zhou Z, Zhang J, Tong J, Cao Y. Adaptive response in mice exposed to 900 MHz radiofrequency fields: primary DNA damage. PLoS One. 7(2):e32040, 2012. (LE, GT, IA)

The phenomenon of adaptive response (AR) in animal and human cells exposed to ionizing radiation is well documented in scientific literature. We have examined whether such AR could be induced in mice exposed to non-ionizing radiofrequency fields (RF) used for wireless communications. Mice were pre-exposed to 900 MHz RF at 120 μW/cm(2) power density for 4 hours/day for 1, 3, 5, 7 and 14 days and then subjected to an acute dose of 3 Gy γ-radiation. The primary DNA damage in the form of alkali labile base damage and single strand breaks in the DNA of peripheral blood leukocytes was determined using the alkaline comet assay. The results indicated that the extent of damage in mice which were pre-exposed to RF for 1 day and then subjected to γ-radiation was similar and not significantly different from those exposed to γ-radiation alone. However, mice which were pre-exposed to RF for 3, 5, 7 and 14 days showed progressively decreased damage and was significantly different from those exposed to γ-radiation alone. Thus, the data indicated that RF pre-exposure is capable of inducing AR and suggested that the pre-exposure for more than 4 hours for 1 day is necessary to elicit such AR.

(NE) Juutilainen J, Heikkinen P, Soikkeli H, Mäki-Paakkanen J. Micronucleus frequency in erythrocytes of mice after long-term exposure to radiofrequency radiation. Int J Radiat Biol. 83(4):213-220, 2007. (LE, GT)

PURPOSE: The aim of the study was to investigate genotoxicity of long-term exposure to radiofrequency (RF) electromagnetic fields by measuring micronuclei in erythrocytes. The blood samples were collected in two animal studies evaluating possible cocarcinogenic effects of RF fields. METHODS: In study A, female CBA/S mice were exposed for 78 weeks (1.5 h/d, 5 d/week) to either a continuous 902.5 MHz signal similar to that emitted by analog NMT (Nordic Mobile Telephone) phones at a whole-body specific absorption rate (SAR) of 1.5 W/kg, or to a pulsed 902.4 MHz signal similar to that of digital GSM (Global System for Mobile Communications) phones at 0.35 W/kg. A third group was sham-exposed, and a fourth group served as cage controls. All but the cage control animals were exposed to 4 Gy of x-rays during three first weeks of the experiment. In study B, female transgenic mice (line K2) and their nontransgenic littermates were exposed for 52 weeks (1.5 h/d, 5 d/week). Two digital mobile phone signals, GSM and DAMPS (Digital Advanced Mobile Phone System), were used at 0.5 W/kg. All but the cage-control animals were exposed 3 times per week to an ultraviolet radiation dose of 1.2 MED (minimum erythema dose). RESULTS AND CONCLUSIONS: The results did not show any effects of RF fields on micronucleus frequency in polychromatic or normochromatic erythrocytes. The results were consistent in two mouse strains (and in a transgenic variant of the second strain), after 52 or 78 weeks of exposure, at three SAR levels relevant to human exposure from mobile phones, and for three different mobile signals.

(E) Karaca E, Durmaz B, Altug H, Yildiz T, Guducu C, Irgi M, Koksal MG, Ozkinay F, Gunduz C, Cogulu O. The genotoxic effect of radiofrequency waves on mouse brain. J Neurooncol 106:53-58, 2012. (GT, GE)

Erratum: J Neurooncol 2012 May;107:665.

Concerns about the health effects of radiofrequency (RF) waves have been raised because of the gradual increase in usage of cell phones, and there are scientific questions and debates about the safety of those instruments in daily life. The aim of this study is to evaluate the genotoxic effects of RF waves in an experimental brain cell culture model. Brain cell cultures of the mice were exposed to 10.715 GHz with specific absorbtion rate (SAR) 0.725 W/kG signals for 6 h in 3 days at 25°C to check for the changes in the micronucleus (MNi) assay and in the expression of 11 proapoptotic and antiapoptotic genes. It was found that MNi rate increased 11-fold and STAT3 expression decreased 7-fold in the cell cultures which were exposed to RF. Cell phones which spread RF may damage DNA and change gene expression in brain cells.

(E) Kesari KK, Behari J. Fifty-gigahertz Microwave exposure effect of radiations on rat brain. Appl Biochem Biotechnol 158:126-139, 2009. (GT, OX, LE)

The object of this study is to investigate the effects of 50-GHz microwave radiation on the brain of Wistar rats. Male rats of the Wistar strain were used in the study. Animals of 60-day age were divided into two groups-group 1, sham-exposed, and group 2, experimental (microwave-exposed). The rats were housed in a temperature-controlled room (25 degrees C) with constant humidity (40-50%) and received food and water ad libitum. During exposure, rats were placed in Plexiglas cages with drilled ventilation holes and kept in an anechoic chamber. The animals were exposed for 2 h a day for 45 days continuously at a power level of 0.86 muW/cm with nominal specific absorption rate 8.0 x 10(-4) w/kg. After the exposure period, the rats were killed and homogenized, and protein kinase C (PKC), DNA double-strand break, and antioxidant enzyme activity [superoxides dismutase (SOD), catalase, and glutathione peroxidase (GPx)] were estimated in the whole brain. Result shows that the chronic exposure to these radiations causes DNA double-strand break (head and tail length, intensity and tail migration) and a significant decrease in GPx and SOD activity (p = <0.05) in brain cells, whereas catalase activity shows significant increase in the exposed group of brain samples as compared with control (p = <0.001). In addition to these, PKC decreased significantly in whole brain and hippocampus ($p < 0.05$). All data are expressed as mean +/- standard deviation. We conclude that these radiations can have a significant effect on the whole brain.

(E) Kesari KK, Behari J, Kumar S. Mutagenic response of 2.45 GHz radiation exposure on rat brain. Int J Radiat Biol 86:334-343, 2010. (GT, OX, LE)

Purpose: To investigate the effect of 2.45 GHz microwave radiation on rat brain of male wistar strain. Material and methods: Male rats of wistar strain (35 days old with 130 +/- 10 g body weight) were selected for this study. Animals were divided into two groups: Sham exposed and experimental. Animals were exposed for 2 h a day for 35 days to 2.45 GHz frequency at 0.34 mW/cm power density. The whole body specific absorption rate (SAR) was estimated to be 0.11 W/Kg. Exposure took place in a ventilated Plexiglas cage and kept in anechoic chamber in a far field configuration from the horn antenna. After the completion of exposure period, rats were sacrificed and the whole brain tissue was dissected and used for study of double strand DNA (Deoxyribonucleic acid) breaks by micro gel electrophoresis and the statistical analysis was carried out using comet assay (IV-2 version software). Thereafter, antioxidant enzymes and histone kinase estimation was also performed. Results: A significant increase was observed in comet head ($P < 0.002$), tail length ($P < 0.0002$) and in tail movement ($P < 0.0001$) in exposed brain cells. An analysis of antioxidant enzymes glutathione peroxidase ($P < 0.005$), and superoxide dismutase ($P < 0.006$) showed a decrease while an increase in catalase ($P < 0.006$) was observed. A significant decrease ($P < 0.023$) in histone kinase was also recorded in the exposed group as compared to the control (sham-exposed) ones. One-way analysis of variance (ANOVA) method was adopted for statistical analysis. Conclusion: The study concludes that the chronic exposure to these radiations may cause significant damage to brain, which may be an indication of possible tumour promotion (Behari and Paulraj 2007).

(E) Khalil AM, Gagaa M, Alshamali A. 8-Oxo-7, 8-dihydro-2'-deoxyguanosine as a biomarker of DNA damage by mobile phone radiation. Hum ExpToxicol 31(7):734-740, 2012. (GT, OX)

We examined the effect of exposure to mobile phone 1800 MHz radio frequency radiation (RFR) upon the urinary excretion of 8-oxo-7, 8-dihydro-2'-deoxyguanosine (8-oxodG), one major form of oxidative DNA damage, in adult male Sprague-Dawley rats. Twenty-four rats were used in three independent experiments (RFR exposed and control, 12 rats, each). The animals were exposed to RFR for 2 h from Global System for Mobile Communications (GSM) signal generator with whole-body-specific absorption rate of 1.0 W/kg. Urine samples were collected from the rat while housed in a metabolic cage during the exposure period over a 4-h period at 0.5, 1.0, 2.0 and 4.0 h from the beginning of exposure. In the control group, the signal generator was left in the turn-off position. The creatinine-standardized concentrations of 8-oxodG were measured. With the exception of the urine collected in the last half an hour of exposure, significant elevations were noticed in the levels of 8-oxodG in urine samples from rats exposed to RFR when compared to control animals. Significant differences were seen overall across time points of urine collection with a maximum at 1 h after exposure, suggesting repair of the DNA lesions leading to 8-oxodG formation.

(E) Kim JY, Hong SY, Lee YM, Yu SA, Koh WS, Hong JR, Son T, Chang SK, Lee M.In vitro assessment of clastogenicity of mobile-phone radiation (835 MHz) using the alkaline comet assay and chromosomal aberration test. Environ Toxicol 23:319-327, 2008. (GT, IA)

Recently we demonstrated that 835-MHz radiofrequency radiation electromagnetic fields (RF-EMF) neither affected the reverse mutation frequency nor accelerated DNA degradation in vitro. Here, two kinds of cytogenetic endpoints were further investigated on mammalian cells exposed to 835-MHz RF-EMF (the most widely used communication frequency band in Korean CDMA mobile phone networks) alone and in combination with model clastogens: in vitro alkaline comet assay and in vitro chromosome aberration (CA) test. No direct cytogenetic effect of 835-MHz RF-EMF was found in the in vitro CA test. The combined exposure of the cells to RF-EMF in the presence of ethylmethanesulfonate (EMS) revealed a weak and insignificant cytogenetic effect when compared to cells exposed to EMS alone in CA test. Also, the comet assay results to evaluate the ability of RF-EMF alone to damage DNA were nearly negative, although showing a small increase in tail moment. However, the applied RF-EMF had potentiation effect in comet assay when administered in combination with model clastogens (cyclophosphamide or 4-nitroquinoline 1-oxide). Thus, our results imply that we cannot confidently exclude any possibility of an increased risk of genetic damage, with important implications for the possible health effects of exposure to 835-MHz electromagnetic fields.

(E) Kumar S, Kesari KK, Behari J. Evaluation of genotoxic effects in male Wistar rats following microwave exposure. Indian J Exp Biol 48:586-592, 2010. (GT, OX)

Wistar rats (70 days old) were exposed for 2 h a day for 45 days continuously at 10 GHz [power density 0.214 mW/cm2, specific absorption rate (SAR) 0.014 W/kg] and 50 GHz (power density 0.86 microW/cm2, SAR 8.0 x10(-4) W/kg). Micronuclei (MN), reactive oxygen species (ROS), and antioxidant enzymes activity were estimated in the blood cells and serum. These radiations induce micronuclei formation and significant increase in ROS production. Significant changes in the level of serum glutathione peroxidase, superoxide dismutase and catalase were observed in exposed group as compared with control group. It is concluded that microwave exposure can be affective at genetic level. This may be an indication of tumor promotion, which comes through the overproduction of reactive oxygen species.

(E) Lakshmi NK, Tiwari R, Bhargava SC, Ahuja YR. Investigations on DNA damage and frequency of micronuclei in occupational exposure to electromagnetic fields (EMFs) emitted from video display terminals (VDTs). Gen MolBiol 33, 154-158, 2010. (GT, HU, LE)

The potential effect of electromagnetic fields (EMFs) emitted from video display terminals (VDTs) to elicit biological response is a major concern for the public. The software professionals are subjected to cumulative EMFs in their occupational environments. This study was undertaken to evaluate DNA damage and incidences of micronuclei in such professionals. To the best of our knowledge, the present study is the first attempt to carry out cytogenetic investigations on assessing bioeffects in personal computer users. The study subjects (n = 138) included software professionals using VDTs for more than 2 years with age, gender, socioeconomic status matched controls (n = 151). DNA damage and frequency of micronuclei were evaluated using alkaline comet assay and cytochalasin blocked micronucleus assay respectively. Overall DNA damage and incidence of micronuclei showed no significant differences between the exposed and control subjects. With exposure characteristics, such as total duration (years) and frequency of use (minutes/day) sub-groups were assessed for such parameters. Although cumulative frequency of use showed no significant changes in the DNA integrity of the classified sub-groups, the long-term users (> 10 years) showed higher induction of DNA damage and increased frequency of micronuclei and micro nucleated cells.

(E) Liu C, Duan W, Xu S, Chen C, He M, Zhang L, Yu Z, Zhou Z. Exposure to 1800 MHz radiofrequency electromagnetic radiation induces oxidative DNA base damage in a mouse spermatocyte-derived cell line. Toxicol Lett 218(1): 2-9, 2013a. (GT, OX, RP)

Whether exposure to radiofrequency electromagnetic radiation (RF-EMR) emitted from mobile phones can induce DNA damage in male germ cells remains unclear. In this study, we conducted a 24 h intermittent exposure (5 min on and 10 min off) of a mouse spermatocyte-derived GC-2 cell line to 1800 MHz Global System for Mobile Communication (GSM) signals in GSM-Talk mode at specific absorption rates (SAR) of 1 W/kg, 2 W/kg or 4 W/kg. Subsequently, through the use of formamidopyrimidine DNA glycosylase (FPG) in a modified comet assay, we determined that the extent of DNA migration was significantly increased at a SAR of 4 W/kg. Flow cytometry analysis demonstrated that levels of the DNA adduct 8-oxoguanine (8-oxoG) were also increased at a SAR of 4 W/kg. These increases were concomitant with similar increases in the generation of reactive oxygen species (ROS); these phenomena were mitigated by co-treatment with the antioxidant α-tocopherol. However, no detectable DNA strand breakage was observed by the alkaline comet assay. Taking together, these findings may imply the novel possibility that RF-EMR with insufficient energy for the direct induction of DNA strand breaks may produce genotoxicity through oxidative DNA base damage in male germ cells.

(E) Liu C, Gao P, Xu SC, Wang Y, Chen CH, He MD, Yu ZP, Zhang L, Zhou Z. Mobile phone radiation induces mode-dependent DNA damage in a mouse spermatocyte-derived cell line: a protective role of melatonin. Int J Radiat Biol. 2013b Aug 19. [Epub ahead of print] (GT, OX, RP)

Purpose: To evaluate whether exposure to mobile phone radiation (MPR) can induce DNA damage in male germ cells. Materials and methods: A mouse spermatocyte-derived GC-2 cell line was exposed to a commercial mobile phone handset once every 20 minutes in standby, listen, dialed or dialing modes for 24 h. DNA damage was determined using an alkaline comet assay. Results: The levels of DNA damage were significantly increased following exposure to MPR in the listen, dialed and dialing modes. Moreover, there were significantly higher increases in the dialed and dialing modes than in the listen mode. Interestingly, these results were consistent with the radiation intensities of these modes. However, the DNA damage effects of MPR in the dialing mode were efficiently attenuated by melatonin pretreatment. Conclusions: These results regarding mode-dependent DNA damage have important implications for the safety of inappropriate mobile phone use by males of reproductive age and also suggest a simple preventive measure, keeping our body from mobile phones as far away as possible, not only during conversations but during "dialed" and "dialing" operation modes as well. Since the "dialed" mode is actually part of the standby mode, mobile phones should be kept at a safe distance from our body even during standby operation. Furthermore, the protective role of melatonin suggests that it may be a promising pharmacological candidate for preventing mobile phone use-related reproductive impairments.

(E) Lixia S, Yao K, Kaijun W, Deqiang L, Huajun H, Xiangwei G, Baohong W, Wei Z, Jianling L, Wei W. Effects of 1.8GHz radiofrequency field on DNA damage and expression of heat shock protein 70 in human lens epithelial cells. Mutat Res 602(1-2):135-42, 2006. (GT, GE)

To investigate the DNA damage, expression of heat shock protein 70 (Hsp70) and cell proliferation of human lens epithelial cells (hLEC) after exposure to the 1.8GHz radiofrequency field (RF) of a global system for mobile communications (GSM). An Xc-1800 RF exposure system was used to employ a GSM signal at 1.8GHz (217Hz amplitude-modulated) with the output power in the specific absorption rate (SAR) of 1, 2 and 3W/kg. After 2h exposure to RF, the DNA damage of hLEC was accessed by comet assay at five different incubation times: 0, 30, 60, 120 and 240min, respectively. Western blot and RT-PCR were used to determine the expression of Hsp70 in hLECs after RF exposure. The proliferation rate of cells was evaluated by bromodeoxyuridine incorporation on days 0, 1 and 4 after exposure. The results show that the difference of DNA-breaks between the exposed and sham-exposed (control) groups induced by 1 and 2W/kg irradiation were not significant at any incubation time point ($P>0.05$). The DNA damage caused by 3W/kg irradiation was significantly increased at the times of 0 and 30min after exposure ($P<0.05$), a phenomenon that could not be seen at the time points of 60, 120 or 240min ($P>0.05$). Detectable mRNA as well as protein expression of Hsp70 was found in all groups. Exposure at SARs of 2 and 3W/kg for 2h exhibited significantly increased Hsp70 protein expression ($P<0.05$), while no change in Hsp70 mRNA expression could be found in any of the groups ($P>0.05$). No difference of the cell proliferation rate between the sham-exposed and exposed cells was found at any exposure dose tested ($P>0.05$). The results indicate that exposure to non-thermal dosages of RF for wireless communications can induce no or repairable DNA damage and the increased Hsp70 protein expression in hLECs occurred without change in the cell proliferation rate. The non-thermal stress response of Hsp70 protein increase to RF exposure might be involved in protecting hLEC from DNA damage and maintaining the cellular capacity for proliferation.

(E) López-Martín E, Bregains J, Relova-Quinteiro JL, Cadarso-Suárez C, Jorge-Barreiro FJ, Ares-Pena FJ. The action of pulse-modulated GSM radiation increases regional changes in brain activity and c-Fos expression in cortical and subcortical areas in a rat

model of picrotoxin-induced seizure proneness. J Neurosci Res. 87(6):1484-1499, 2009. (AS, GE, WS, IA)

The action of the pulse-modulated GSM radiofrequency of mobile phones has been suggested as a physical phenomenon that might have biological effects on the mammalian central nervous system. In the present study, GSM-exposed picrotoxin-pretreated rats showed differences in clinical and EEG signs, and in c-Fos expression in the brain, with respect to picrotoxin-treated rats exposed to an equivalent dose of unmodulated radiation. Neither radiation treatment caused tissue heating, so thermal effects can be ruled out. The most marked effects of GSM radiation on c-Fos expression in picrotoxin-treated rats were observed in limbic structures, olfactory cortex areas and subcortical areas, the dentate gyrus, and the central lateral nucleus of the thalamic intralaminar nucleus group. Nonpicrotoxin-treated animals exposed to unmodulated radiation showed the highest levels of neuronal c-Fos expression in cortical areas. These results suggest a specific effect of the pulse modulation of GSM radiation on brain activity of a picrotoxin-induced seizure-proneness rat model and indicate that this mobile-phone-type radiation might induce regional changes in previous preexcitability conditions of neuronal activation.

(E) Luukkonen J, Hakulinen P, Mäki-Paakkanen J, Juutilainen J, Naarala J. Enhancement of chemically induced reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells by 872MHz radiofrequency radiation. Mutat Res 662:54-58, 2009. (GT, OX, WS)

The objective of the study was to investigate effects of 872 MHz radiofrequency (RF) radiation on intracellular reactive oxygen species (ROS) production and DNA damage at a relatively high SAR value (5W/kg). The experiments also involved combined exposure to RF radiation and menadione, a chemical inducing intracellular ROS production and DNA damage. The production of ROS was measured using the fluorescent probe dichlorofluorescein and DNA damage was evaluated by the Comet assay. Human SH-SY5Y neuroblastoma cells were exposed to RF radiation for 1h with or without menadione. Control cultures were sham exposed. Both continuous waves (CW) and a pulsed signal similar to that used in global system for mobile communications (GSM) mobile phones were used. Exposure to the CW RF radiation increased DNA breakage ($p<0.01$) in comparison to the cells exposed only to menadione. Comparison of the same groups also showed that ROS level was higher in cells exposed to CW RF radiation at 30 and 60 min after the end of exposure ($p<0.05$ and $p<0.01$, respectively). No effects of the GSM signal were seen on either ROS production or DNA damage. The results of the present study suggest that 872MHz CW RF radiation at 5W/kg might enhance chemically induced ROS production and thus cause secondary DNA damage. However, there is no known mechanism that would explain such effects from CW RF radiation but not from GSM modulated RF radiation at identical SAR.

(NE) Luukkonen J, Juutilainen J, Naarala J. Combined effects of 872 MHz radiofrequency radiation and ferrous chloride on reactive oxygen species production and DNA damage in human SH-SY5Y neuroblastoma cells. Bioelectromagnetics 31:417-424, 2010. (GT, OX)

The aim of the present study was to investigate possible cooperative effects of radiofrequency (RF) radiation and ferrous chloride (FeCl) on reactive oxygen species (ROS) production and

DNA damage. In order to test intracellular ROS production as a possible underlying mechanism of DNA damage, we applied the fluorescent probe DCFH-DA. Integrity of DNA was quantified by alkaline comet assay. The exposures to 872 MHz RF radiation were conducted at a specific absorption rate (SAR) of 5 W/kg using continuous waves (CW) or a modulated signal similar to that used in Global System for Mobile Communications (GSM) phones. Four groups were included: Sham exposure (control), RF radiation, Chemical treatment, Chemical treatment, and RF radiation. In the ROS production experiments, human neuroblastoma (SH-SY5Y) cells were exposed to RF radiation and 10 microg/ml FeCl for 1 h. In the comet assay experiments, the exposure time was 3 h and an additional chemical (0.015% diethyl maleate) was used to make DNA damage level observable. The chemical treatments resulted in statistically significant responses, but no effects from either CW or modulated RF radiation were observed on ROS production, DNA damage or cell viability.

(NE) Maes A, Van Gorp U, Verschaeve L. Cytogenetic investigation of subjects professionally exposed to radiofrequency radiation. Mutagenesis 21:139-42, 2006. (GT, IA)

Nowadays, virtually everybody is exposed to radiofrequency radiation (RFR) from mobile phone base station antennas or other sources. At least according to some scientists, this exposure can have detrimental health effects. We investigated cytogenetic effects in peripheral blood lymphocytes from subjects who were professionally exposed to mobile phone electromagnetic fields in an attempt to demonstrate possible RFR-induced genetic effects. These subjects can be considered well suited for this purpose as their RFR exposure is 'normal' though rather high, and definitely higher than that of the 'general population'. The alkaline comet assay, sister chromatid exchange (SCE) and chromosome aberration tests revealed no evidence of RFR-induced genetic effects. Blood cells were also exposed to the well known chemical mutagen mitomycin C in order to investigate possible combined effects of RFR and the chemical. No cooperative action was found between the electromagnetic field exposure and the mutagen using either the comet assay or SCE test.

(E) Manti L, Braselmann H, Calabrese ML, Massa R, Pugliese M, Scampoli P, Sicignano G, Grossi G. Effects of modulated microwave radiation at cellular telephone frequency (1.95 GHz) on X-ray-induced chromosome aberrations in human lymphocytes in vitro. Radiat Res 169:575-583, 2008. (GT, IA)

The case for a DNA-damaging action produced by radiofrequency (RF) signals remains controversial despite extensive research. With the advent of the Universal Mobile Telecommunication System (UMTS) the number of RF-radiation-exposed individuals is likely to escalate. Since the epigenetic effects of RF radiation are poorly understood and since the potential modifications of repair efficiency after exposure to known cytotoxic agents such as ionizing radiation have been investigated infrequently thus far, we studied the influence of UMTS exposure on the yield of chromosome aberrations induced by X rays. Human peripheral blood lymphocytes were exposed in vitro to a UMTS signal (frequency carrier of 1.95 GHz) for 24 h at 0.5 and 2.0 W/kg specific absorption rate (SAR) using a previously characterized waveguide system. The frequency of chromosome aberrations was measured on metaphase spreads from cells given 4 Gy of X rays immediately before RF radiation or sham exposures by fluorescence in situ hybridization. Unirradiated controls were RF-radiation- or sham-exposed. No significant variations due to the UMTS exposure were found in the fraction of aberrant cells. However, the frequency of exchanges per cell was affected by the SAR, showing a small but statistically significant increase of 0.11 exchange per cell compared to 0 W/kg SAR. We conclude that, although the 1.95 GHz signal (UMTS modulated) does not exacerbate the yield of aberrant cells caused by ionizing radiation, the overall burden of X-ray-induced chromosomal damage per cell in first-mitosis lymphocytes may be enhanced at 2.0 W/kg SAR. Hence the SAR may either influence the repair of X-ray-induced DNA breaks or alter the cell death pathways of the damage response.

(E) Mazor R, Korenstein-Ilan A, Barbul A, Eshet Y, Shahadi A, Jerby E, Korenstein R. Increased levels of numerical chromosome aberrations after in vitro exposure of human peripheral blood lymphocytes to radiofrequency electromagnetic fields for 72 hours. Radiat Res. 169(1):28-37, 2008. (GT)

We investigated the effects of 72 h in vitro exposure of 10 human lymphocyte samples to radiofrequency electromagnetic fields (800 MHz, continuous wave) on genomic instability. The lymphyocytes were exposed in a specially designed waveguide resonator at specific absorption rates (SARs) of 2.9 and 4.1 W/kg in a temperature range of 36-37 degrees C. The induced aneuploidy of chromosomes 1, 10, 11 and 17 was determined by interphase FISH using semi-automated image analysis. We observed increased levels of aneuploidy depending on the chromosome studied as well as on the level of exposure. In chromosomes 1 and 10, there was increased aneuploidy at the higher SAR, while for chromosomes 11 and 17, the increases were observed only for the lower SAR. Multisomy (chromosomal gains) appeared to be the primary contributor to the increased aneuploidy. The effect of temperature on the level of aneuploidy was examined over the range of 33.5-40 degrees C for 72 h with no statistically significant difference in the level of aneuploidy compared to 37 degrees C. These findings suggest the possible existence of an athermal effect of RF radiation that causes increased levels of aneuploidy. These results contribute to the assessment of potential health risks after continuous chronic exposure to RF radiation at SARs close to the current levels set by ICNIRP guidelines.

(E) Nikolova T, Czyz J, Rolletschek A, Blyszczuk P, Fuchs J, Jovtchev G, Schuderer J, Kuster N, Wobus AM. Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. ASEB J 19(12):1686-1688, 2005. (GT, GE)

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

(E) Nittby H, Widegren B, Krogh M, Grafström G, Berlin H, Rehn G, Eberhardt JL, Malmgren L, Persson BRR, Salford L. Exposure to radiation from global system for mobile communications at 1,800 MHz significantly changes gene expression in rat hippocampus and cortex. Environmentalist 28(4), 458-465, 2008. (GE)

We have earlier shown that radio frequency electromagnetic fields can cause significant leakage of albumin through the blood–brain barrier of exposed rats as compared to non-exposed rats, and also significant neuronal damage in rat brains several weeks after a 2 h exposure to a mobile phone, at 915 MHz with a global system for mobile communications (GSM) frequency modulation, at whole-body specific absorption rate values (SAR) of 200, 20, 2, and 0.2 mW/kg. We have now studied whether 6 h of exposure to the radiation from a GSM mobile test phone at 1,800 MHz (at a whole-body SAR-value of 13 mW/kg, corresponding to a brain SAR-value of 30 mW/kg) has an effect upon the gene expression pattern in rat brain cortex and hippocampus—areas where we have observed albumin leakage from capillaries into neurons and neuronal damage. Microarray analysis of 31,099 rat genes, including splicing variants, was performed in cortex and hippocampus of 8 Fischer 344 rats, 4 animals exposed to global system for mobile communications electromagnetic fields for 6 h in an anechoic chamber, one rat at a time, and 4 controls kept as long in the same anechoic chamber without exposure, also in this case one rat at a time. Gene ontology analysis (using the gene ontology categories biological processes, molecular functions, and cell components) of the differentially expressed genes of the exposed animals versus the control group revealed the following highly significant altered gene categories in both cortex and hippocampus: extracellular region, signal transducer activity, intrinsic to membrane, and integral to membrane. The fact that most of these categories are connected with membrane functions may have a relation to our earlier observation of albumin transport through brain capillaries.

(E) Nylund R, Leszczynski D. Mobile phone radiation causes changes in gene and protein expression in human endothelial cell lines and the response seems to be genome- and proteome-dependent. Proteomics 6:4769-4780, 2006. (GE, CS)

We have examined in vitro cell response to mobile phone radiation (900 MHz GSM signal) using two variants of human endothelial cell line: EA.hy926 and EA.hy926v1. Gene expression changes were examined in three experiments using cDNA Expression Arrays and protein expression changes were examined in ten experiments using 2-DE and PDQuest software. Obtained results show that gene and protein expression were altered, in both examined cell lines, in response to one hour mobile phone radiation exposure at an average specific absorption rate of 2.8 W/kg. However, the same genes and proteins were differently affected by the exposure in each of the cell lines. This suggests that the cell response to mobile phone radiation might be genome- and proteome-dependent. Therefore, it is likely that different types of cells and from different species might respond differently to mobile phone radiation or might have different sensitivity to this weak stimulus. Our findings might also explain, at least in part, the origin of discrepancies in replication studies between different laboratories.

(E) Panagopoulos DJ, Chavdoula ED, Nezis IP, Margaritis LH. Cell death induced by GSM 900-MHz and DCS 1800-MHz mobile telephony radiation. Mutat Res 626:69-78, 2007. (GT, RP)

In the present study, the TUNEL (Terminal deoxynucleotidetransferasedUTP Nick End Labeling) assay - a well known technique widely used for detecting fragmented DNA in various types of cells - was used to detect cell death (DNA fragmentation) in a biological model, the early and mid stages of oogenesis of the insect Drosophila melanogaster. The flies were exposed in vivo to either GSM 900-MHz (Global System for Mobile telecommunications) or DCS 1800-MHz (Digital Cellular System) radiation from a common digital mobile phone, for few minutes per day during the first 6 days of their adult life. The exposure conditions were similar to those to which a mobile phone user is exposed, and were determined according to previous studies of ours [D.J Panagopoulos, A. Karabarbounis, L.H. Margaritis, Effect of GSM 900-MHz mobile phone radiation on the reproductive capacity of D. melanogaster, Electromagn. Biol Med 23 (2004) 29-43; D.J Panagopoulos, N. Messini, A. Karabarbounis, A.L. Philippetis, L.H. Margaritis, Radio frequency electromagnetic radiation within "safety levels" alters the physiological function of insects, in: P. Kostarakis, P. Stavroulakis (Eds.), Proceedings of the Millennium International Workshop on Biological Effects of Electromagnetic Fields, Heraklion, Crete, Greece, October 17-20, 2000, pp. 169-175, ISBN: 960-86733-0-5; D.J Panagopoulos, L.H. Margaritis, Effects of electromagnetic fields on the reproductive capacity of D. melanogaster, in: P. Stavroulakis (Ed.), Biological Effects of Electromagnetic Fields, Springer, 2003, pp. 545-578], which had shown a large decrease in the oviposition of the same insect caused by GSM radiation. Our present results suggest that the decrease in oviposition previously reported, is due to degeneration of large numbers of egg chambers after DNA fragmentation of their constituent cells, induced by both types of mobile telephony radiation. Induced cell death is recorded for the first time, in all types of cells constituting an egg chamber (follicle cells, nurse cells and the oocyte) and in all stages of the early and mid-oogenesis, from germarium to stage 10, during which programmed cell death does not physiologically occur. Germarium and stages 7-8 were found to be the most sensitive developmental stages also in response to electromagnetic stress induced by the GSM and DCS fields and, moreover, germarium was found to be even more sensitive than stages 7-8.

(NE) Paparini A, Rossi P, Gianfranceschi G, Brugaletta V, Falsaperla R, De Luca P, Romano Spica V. No evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal. Bioelectromagnetics. 29(4):312-323, 2008. (GE)

To analyze possible effects of microwaves on gene expression, mice were exposed to global system for mobile communication (GSM) 1800 MHz signal for 1 h at a whole body SAR of 1.1 W/kg. Gene expression was studied in the whole brain, where the average SAR was 0.2 W/kg, by expression microarrays containing over 22,600 probe sets. Comparison of data from sham and exposed animals showed no significant difference in gene expression modulation. However, when less stringent constraints were adopted to analyze microarray results, 75 genes were found to be modulated following exposure. Forty-two probes showed fold changes ranging from 1.5 to 2.8, whereas 33 were down-regulated from 0.67- to 0.29-fold changes, but these differences in gene expression were not confirmed by real-time PCR. Under these specific limited conditions, no consistent indication of gene expression modulation in whole mouse brain was found associated to GSM 1800 MHz exposure.

(E) Paulraj R, Behari J. Single strand DNA breaks in rat brain cells exposed to microwave radiation. Mutat Res 596:76-80, 2006. (GT, LE)

This investigation concerns with the effect of low intensity microwave (2.45 and 16.5GHz, SAR 1.0 and 2.01W/kg, respectively) radiation on developing rat brain. Wistar rats (35 days old, male, six rats in each group) were selected for this study. These animals were exposed for 35 days at the above mentioned frequencies separately in two different exposure systems. After the exposure period, the rats were sacrificed and the whole brain tissue was dissected and used for study of single strand DNA breaks by micro gel electrophoresis (comet assay). Single strand DNA breaks were measured as tail length of comet. Fifty cells from each slide and two slides per animal were observed. One-way ANOVA method was adopted for statistical analysis. This study shows that the chronic exposure to these radiations cause statistically significant ($p<0.001$) increase in DNA single strand breaks in brain cells of rat.

(E) Pesnya DS, Romanovsky AV. Comparison of cytotoxic and genotoxic effects of plutonium-239 alpha particles and mobile phone GSM 900 radiation in the Allium cepa test. Mutat Res. 2012 Oct 8. pii: S1383-5718(12)00291-4. doi: 10.1016/j.mrgentox.2012.08.010. [Epub ahead of print] (GT)

The goal of this study was to compare the cytotoxic and genotoxic effects of plutonium-239 alpha particles and GSM 900 modulated mobile phone radiation in the Allium cepa test. Three groups of bulbs were exposed to mobile phone radiation during 0 (sham), 3 and 9hours. A positive control group was treated during 20 min with plutonium-239 alpha-radiation. Mitotic abnormalities, chromosome aberrations, micronuclei and mitotic index were analyzed. Exposure to alpha-radiation from plutonium-239 and exposure to modulated radiation from mobile phone during 3 and 9h significantly increased the mitotic index. GSM 900 mobile phone radiation as well as alpha-radiation from plutonium-239 induced both clastogenic and aneugenic effects. However, the aneugenic activity of mobile phone radiation was more pronounced. After 9 hours of exposure to mobile phone radiation, polyploid cells, three-groups metaphases, amitoses and some unspecified abnormalities were detected, which were not registered in the other experimental groups. Importantly, GSM 900 mobile phone radiation increased the mitotic index, the frequency of mitotic and chromosome abnormalities, and the micronucleus frequency in a time-dependent manner. Due to its sensitivity, the Allium cepa test can be recommended as a useful cytogenetic assay to assess cytotoxic and genotoxic effects of radiofrequency electromagnetic fields.

(NE) Qutob SS, Chauhan V, Bellier PV, Yauk CL, Douglas GR, Berndt L, Williams A, Gajda GB, Lemay E, Thansandote A, McNamee JP. Microarray gene expression profiling of a human glioblastoma cell line exposed in vitro to a 1.9 GHz pulse-modulated radiofrequency field. Radiat Res 165:636-644, 2006. (GE)

The widespread use of mobile phones has led to public concerns about the health effects associated with exposure to radiofrequency (RF) fields. The paramount concern of most persons relates to the potential of these fields to cause cancer. Unlike ionizing radiation, RF fields used for mobile telecommunications (800-1900 MHz) do not possess sufficient energy to directly damage DNA. Most rodent bioassay and in vitro genotoxicity/mutation studies have reported that RF fields at non-thermal levels have no direct mutagenic, genotoxic or carcinogenic effects.

However, some evidence has suggested that RF fields may cause detectable postexposure changes in gene expression. Therefore, the purpose of this study was to assess the ability of exposure to a 1.9 GHz pulse-modulated RF field for 4 h at specific absorption rates (SARs) of 0.1, 1.0 and 10.0 W/kg to affect global gene expression in U87MG glioblastoma cells. We found no evidence that non-thermal RF fields can affect gene expression in cultured U87MG cells relative to the nonirradiated control groups, whereas exposure to heat shock at 43 degrees C for 1 h up-regulated a number of typical stress-responsive genes in the positive control group. Future studies will assess the effect of RF fields on other cell lines and on gene expression in the mouse brain after in vivo exposure.

(E) Remondini D, Nylund R, Reivinen J, Poulletier de Gannes F, Veyret B, Lagroye I, Haro E, Trillo MA, Capri M, Franceschi C, Schlatterer K, Gminski R, Fitzner R, Tauber R, Schuderer J, Kuster N, Leszczynski D, Bersani F, Maercker C. Gene expression changes in human cells after exposure to mobile phone microwaves. Proteomics 6:4745-4754, 2006. (GE, CS)

Possible biological effects of mobile phone microwaves were investigated in vitro. In this study, which was part of the 5FP EU project REFLEX (Risk Evaluation of Potential Environmental Hazards From Low-Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods), six human cell types, immortalized cell lines and primary cells, were exposed to 900 and 1800 MHz. RNA was isolated from exposed and sham-exposed cells and labeled for transcriptome analysis on whole-genome cDNA arrays. The results were evaluated statistically using bioinformatics techniques and examined for biological relevance with the help of different databases. NB69 neuroblastoma cells, T lymphocytes, and CHME5 microglial cells did not show significant changes in gene expression. In EA.hy926 endothelial cells, U937 lymphoblastoma cells, and HL-60 leukemia cells we found between 12 and 34 up- or down-regulated genes. Analysis of the affected gene families does not point towards a stress response. However, following microwave exposure, some but not all human cells might react with an increase in expression of genes encoding ribosomal proteins and therefore up-regulating the cellular metabolism.

(NE) Ros-Llor I, Sanchez-Siles M, Camacho-Alonso F, Lopez-Jornet P. Effect of mobile phones on micronucleus frequency in human exfoliated oral mucosal cells. Oral Dis. 18:786-792, 2012. (GT)

Objective: In the last two decades, the use of mobile phones has increased enormously all over the world. The controversy regarding whether radiofrequency (RF) fields exert effects upon biological systems is a concern for the general population. An evaluation is made of DNA damage and cytokinetic defects, proliferative potential, and cell death because of RF radiation emitted by mobile phones in healthy young users. Study design: This cohort study was carried out in 50 Caucasian mobile phone users. We collected two cell samples from each subject (a total of 100 cell samples), corresponding to the right and left cheek mucosa, respectively. Case histories and personal information were assessed, including age, gender, body height and weight, history of cancer, smoking and alcohol consumption, exposure to chemical carcinogens or radiation, and dietary habits. Sampling comprised cell collection from both cheeks with a cytobrush, centrifugation, slide preparation, fixation, and staining, followed by fluorescent microscopic analysis. A total of 2000 exfoliated cells were screened for nuclear abnormalities, especially micronucleus. Results: No statistically significant changes were recorded in relation to age, gender, body mass index, or smoking status. A comparison of the results vs the control area according to the side of the face on which the mobile phone was placed, and in relation to the duration of exposure (years) to mobile phone radiation in the total 100 samples, yielded no significant differences. Conclusions: No genotoxic effects because of RF exposure were observed in relation to any of the study parameters.

(NE) Sakuma N, Komatsubara Y, Takeda H, Hirose H, Sekijima M, Nojima T, Miyakoshi J. DNA strand breaks are not induced in human cells exposed to 2.1425 GHz band CW and W-CDMA modulated radiofrequency fields allocated to mobile radio base stations. Bioelectromagnetics 27:51-57, 2006. (CT)

We conducted a large-scale in vitro study focused on the effects of low level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system in order to test the hypothesis that modulated RF fields may act as a DNA damaging agent. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced different levels of DNA damage. Human glioblastoma A172 cells and normal human IMR-90 fibroblasts from fetal lungs were exposed to mobile communication frequency radiation to investigate whether such exposure produced DNA strand breaks in cell culture. A172 cells were exposed to W-CDMA radiation at SARs of 80, 250, and 800 mW/kg and CW radiation at 80 mW/kg for 2 and 24 h, while IMR-90 cells were exposed to both W-CDMA and CW radiations at a SAR of 80 mW/kg for the same time periods. Under the same RF field exposure conditions, no significant differences in the DNA strand breaks were observed between the test groups exposed to W-CDMA or CW radiation and the sham exposed negative controls, as evaluated immediately after the exposure periods by alkaline comet assays. Our results confirm that low level exposures do not act as a genotoxicant up to a SAR of 800 mW/kg.

(NE) Sakurai T, Kiyokawa T, Narita E, Suzuki Y, Taki M, Miyakoshi J. Analysis of gene expression in a human-derived glial cell line exposed to 2.45 GHz continuous radiofrequency electromagnetic fields. J Radiat Res. 52(2):185-192, 2011. (GE)

The increasing use of mobile phones has aroused public concern regarding the potential health risks of radiofrequency (RF) fields. We investigated the effects of exposure to RF fields (2.45 GHz, continuous wave) at specific absorption rate (SAR) of 1, 5, and 10 W/kg for 1, 4, and 24 h on gene expression in a normal human glial cell line, SVGp12, using DNA microarray. Microarray analysis revealed 23 assigned gene spots and 5 non-assigned gene spots as prospective altered gene spots. Twenty-two genes out of the 23 assigned gene spots were further analyzed by reverse transcription-polymerase chain reaction to validate the results of microarray, and no significant alterations in gene expression were observed. Under the experimental conditions used in this study, we found no evidence that exposure to RF fields affected gene expression in SVGp12 cells.

(NE) Sannino A, Di Costanzo G, Brescia F, Sarti M, Zeni O, Juutilainen J, Scarfì MR. Human fibroblasts and 900 MHz radiofrequency radiation: evaluation of DNA damage after exposure and co-exposure to 3-Chloro-4-(dichloromethyl)-5-Hydroxy-2(5h)-furanone (MX). Radiat Res 171:743-751, 2009. (NT, IA)

Abstract Sannino, A., Di Costanzo, G., Brescia, F., Sarti, M., Zeni, O., Juutilainen, J and Scarfì, M. R. Human Fibroblasts and 900 MHz Radiofrequency Radiation: Evaluation of DNA Damage after Exposure and Co-exposure to 3-Chloro-4-(dichloromethyl)-5-Hydroxy-2(5h)-furanone (MX). Radiat Res 171, 743-751 (2009). The aim of this study was to investigate DNA damage in human dermal fibroblasts from a healthy subject and from a subject affected by Turner's syndrome that were exposed for 24 h to radiofrequency (RF) radiation at 900 MHz. The RF-radiation exposure was carried out alone or in combination with 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX), a well-known environmental mutagen and carcinogen produced during the chlorination of drinking water. Turner's syndrome fibroblasts were also exposed for a shorter time (1 h). A signal similar to that emitted by Global System for Mobile Communications (GSM) mobile phones was used at a specific absorption rate of 1 W/kg under strictly controlled conditions of temperature and dosimetry. To evaluate DNA damage after RF-radiation exposure alone, the alkaline comet assay and the cytokinesis-block micronucleus assay were used. In the combined-exposure experiments, MX was given at a concentration of 25 microM for 1 h immediately after the RF-radiation exposure, and the effects were evaluated by the alkaline comet assay. The results revealed no genotoxic and cytotoxic effects from RF radiation alone in either cell line. As expected, MX treatment induced an increase in DNA migration in the comet assay, but no enhancement of the MX-induced DNA damage was observed in the cells exposed to RF radiation.

(E) Schwarz C, Kratochvil E, Pilger A, Kuster N, Adlkofer F, Rüdiger HW. Radiofrequency electromagnetic fields (UMTS, 1,950 MHz) induce genotoxic effects in vitro in human fibroblasts but not in lymphocytes. Int Arch Occup Environ Health 81:755-767, 2008. (GT, CS)

OBJECTIVE: Universal Mobile Telecommunication System (UMTS) was recently introduced as the third generation mobile communication standard in Europe. This was done without any information on biological effects and genotoxic properties of these particular high-frequency electromagnetic fields. This is discomforting, because genotoxic effects of the second generation standard Global System for Mobile Communication have been reported after exposure of human cells in vitro. METHODS: Human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures were exposed to 1,950 MHz UMTS below the specific absorption rate (SAR) safety limit of 2 W/kg. The alkaline comet assay and the micronucleus assay were used to ascertain dose and time-dependent genotoxic effects. Five hundred cells per slide were visually evaluated in the comet assay and comet tail factor (CTF) was calculated. In the micronucleus assay 1,000 binucleated cells were evaluated per assay. The origin of the micronuclei was determined by fluorescence labeled anticentromere antibodies. All evaluations were performed under blinded conditions. RESULTS: UMTS exposure increased the CTF and induced centromere-negative micronuclei (MN) in human cultured fibroblasts in a dose and time-dependent way. Incubation for 24 h at a SAR of 0.05 W/kg generated a statistically significant rise in both CTF and MN (P = 0.02). At a SAR of 0.1 W/kg the CTF was significantly increased after 8 h of incubation (P = 0.02), the number of MN after 12 h (P = 0.02). No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with Phytohemagglutinin. CONCLUSION: UMTS exposure may cause genetic alterations in some but not in all human cells in vitro.

(E) Sekeroğlu V, Akar A, Sekeroğlu ZA. Cytotoxic and genotoxic effects of high-frequency electromagnetic fields (GSM 1800 MHz) on immature and mature rats. Ecotoxicol Environ Saf. 80:140-144, 2012. (LE, GT, DE)

We investigated the cytogenotoxic effects of high frequency electromagnetic fields (HF-EMF) for 45 day and the effect of a recovery period of 15 day after exposure to EMF on bone marrow cells of immature and mature rats. The animals in treatment groups were exposed to 1800 MHz EMF at SAR of 0.37 W/kg and 0.49 W/kg for 2h/day for 45 day. Two recovery groups were kept for a recovery period of 15 day without EMF after exposure to HF-EMF. Two control groups for both immature and mature rats were also included. Significant differences were also observed in chromosome aberrations (CA), micronucleus (MN) frequency, mitotic index (MI) and ratio of polychromatic erythrocytes (PCEs) in all treatment groups. The cytogenotoxic damage was more remarkable in immature rats and, the recovery period did not improve this damage in immature rats. Because much higher and irreversible cytogenotoxic damage was observed in immature rats than in mature rats, further studies are needed to understand effects of EMF on DNA damage and DNA repair, and to determine safe limits for environment and human, especially for children.

(NE) Sekijima M, Takeda H, Yasunaga K, Sakuma N, Hirose H, Nojima T, Miyakoshi J. 2-GHz band CW and W-CDMA modulated radiofrequency fields have no significant effect on cell proliferation and gene expression profile in human cells. J Radiat Res. 51(3):277-284, 2010. (GE)

We investigated the mechanisms by which radiofrequency (RF) fields exert their activity, and the changes in both cell proliferation and the gene expression profile in the human cell lines, A172 (glioblastoma), H4 (neuroglioma), and IMR-90 (fibroblasts from normal fetal lung) following exposure to 2.1425 GHz continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) RF fields at three field levels. During the incubation phase, cells were exposed at the specific absorption rates (SARs) of 80, 250, or 800 mW/kg with both CW and W-CDMA RF fields for up to 96 h. Heat shock treatment was used as the positive control. No significant differences in cell growth or viability were observed between any test group exposed to W-CDMA or CW radiation and the sham-exposed negative controls. Using the Affymetrix Human Genome Array, only a very small (< 1%) number of available genes (ca. 16,000 to 19,000) exhibited altered expression in each experiment. The results confirm that low-level exposure to 2.1425 GHz CW and W-CDMA RF fields for up to 96 h did not act as an acute cytotoxicant in either cell proliferation or the gene expression profile. These results suggest that RF exposure up to the limit of whole-body average SAR levels as specified in the ICNIRP guidelines is unlikely to elicit a general stress response in the tested cell lines under these conditions.

(E) Souza LD, Cerqueira ED, Meireles JR. Assessment of nuclear abnormalities in exfoliated cells from the oral epithelium of mobile phone users. Electromagn Biol Med. 2013 May 28. [Epub ahead of print] (GE, HU)

Abstract Transmission and reception of mobile telephony signals take place through electromagnetic wave radiation, or electromagnetic radiofrequency fields, between the mobile terminal and the radio base station. Based on reports in the literature on adverse effects from exposure to this type of radiation, the objective of this study was to evaluate the genotoxic and cytotoxic potential of such exposure, by means of the micronucleus test on exfoliated cells from the oral epithelium. The sample included 45 individuals distributed in 3 groups according to the amount of time in hours per week (t) spent using mobile phones: group I, $t > 5$ h; group II, $t > 1$ h and ≤ 5 h; and group III, $t \leq 1$ h. Cells from the oral mucosa were analyzed to assess the numbers of micronuclei, broken egg structures and degenerative nuclear abnormalities indicative of apoptosis (condensed chromatin, karyorrhexis and pyknosis) or necrosis (karyolysis in addition to these changes). The occurrences of micronuclei and degenerative nuclear abnormalities did not differ between the groups, but the number of broken egg (structures that may be associated with gene amplification) was significantly greater in the individuals in group I ($p < 0.05$).

(NE) Speit G, Schütz P, Hoffmann H. Genotoxic effects of exposure to radiofrequency electromagnetic fields (RF-EMF) in cultured mammalian cells are not independently reproducible. Mutat Res. 626(1-2):42-47, 2007. (GT)

Conflicting results have been published regarding the induction of genotoxic effects by exposure to radiofrequency electromagnetic fields (RF-EMF). Using the comet assay, the micronucleus test and the chromosome aberration test with human fibroblasts (ES1 cells), the EU-funded "REFLEX" project (Risk Evaluation of Potential Environmental Hazards From Low Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods) reported clearly positive effects for various exposure conditions. Because of the ongoing discussion on the biological significance of the effects observed, it was the aim of the present study to independently repeat the results using the same cells, the same equipment and the same exposure conditions. We therefore exposed ES1 cells to RF-EMF (1800 MHz; SAR 2 W/kg, continuous wave with intermittent exposure) for different time periods and then performed the alkaline (pH>13) comet assay and the micronucleus test (MNT). For both tests, clearly negative results were obtained in independently repeated experiments. We also performed these experiments with V79 cells, a sensitive Chinese hamster cell line that is frequently used in genotoxicity testing, and also did not measure any genotoxic effect in the comet assay and the MNT. Appropriate measures of quality control were considered to exclude variations in the test performance, failure of the RF-EMF exposure or an evaluation bias. The reasons for the difference between the results reported by the REFLEX project and our experiments remain unclear.

(NE) Stronati L, Testa A, Moquet J, Edwards A, Cordelli E, Villani P, Marino C, Fresegna AM, Appolloni M, Lloyd D. 935 MHz cellular phone radiation. An in vitro study of genotoxicity in human lymphocytes. Int J Radiat Biol 82:339-346, 2006. (GT, IA)

Purpose: The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with x-rays was investigated in vitro using several assays on human lymphocytes. The chosen specific absorption rate (SAR) values are near the upper limit of actual energy absorption in localized tissue when persons use some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and established mutagen.Methods: Blood specimens from 14 donors were exposed continuously for 24 h to a

Global System for Mobile Communications (GSM) basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp x-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of in vitro cell cycling.Results: By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition RFR did not modify any measured effects of the x-radiation. Conclusions: This study has used several standard in vitro tests for chromosomal and DNA damage in Go human lymphocytes exposed in vitro to a combination of x-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic per se or whether, it can influence the genotoxicity of the well-established clastogenic agent; x-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed.

(E) Sun LX, Yao K, He JL, Lu DQ, Wang KJ, Li HW.[Effect of acute exposure to microwave from mobile phone on DNA damage and repair of cultured human lens epithelial cells in vitro.] Zhonghua Lao Dong Wei Sheng Zhi Ye Bing ZaZhi. 24:465-467, 2006. [Article in Chinese] (GT)

OBJECTIVE: To investigate the DNA damage of human lens epithelial cells (LECs) caused by acute exposure to low-power 217 Hz modulated 1.8 GHz microwave radiation and DNA repair. METHODS: Cultured LECs were exposed to 217 Hz modulated 1.8 GHz microwave radiation at SAR (specific absorption rate) of 0, 1, 2, 3 and 4 W/kg for 2 hours in an sXc-1800 incubator and irradiate system. The DNA single strand breaks were detected with comet assay in sham-irradiated cells and irradiated cells incubated for varying periods: 0, 30, 60, 120 and 240 min after irradiation. Images of comets were digitized and analyzed using an Imagine-pro plus software, and the indexes used in this study were tail length (TL) and tail moment (TM). RESULTS: The difference in DNA-breaks between the exposure and sham exposure groups induced by 1 and 2 W/kg irradiation was not significant at every detect time ($P > 0.05$). As for the dosage of 3 and 4 W/kg there was difference in both groups immediately after irradiation ($P < 0.01$). At the time of 30 min after irradiation the difference went on at both group ($P < 0.01$). However, the difference disappeared after one hour's incubation in 3 W/kg group ($P > 0.05$), and existed in 4 W/kg group. CONCLUSION: No or repairable DNA damage was observed after 2 hour irradiation of 1.8 GHz microwave on LECs when SAR </= 3 W/kg. The DNA damages caused by 4 W/kg irradiation were irreversible.

(E) Tiwari R, Lakshmi NK, Surender V, Rajesh AD, Bhargava SC, Ahuja YR. Combinative exposure effect of radio frequency signals from CDMA mobile phones and aphidicolin on DNA integrity. Electromagn Biol Med 27:418-425, 2008. (GT, IA)

The aim of present study is to assess DNA integrity on the effect of exposure to a radio frequency (RF) signal from Code Division Multiple Access (CDMA) mobile phones. Whole blood samples from six healthy male individuals were exposed for RF signals from a CDMA mobile phone for 1 h. Alkaline comet assay was performed to assess the DNA damage. The combinative exposure effect of the RF signals and APC at two concentrations on DNA integrity was studied. DNA repair efficiency of the samples was also studied after 2 h of exposure. The

RF signals and APC (0.2 microg/ml) alone or in synergism did not have any significant DNA damage as compared to sham exposed. However, univariate analysis showed that DNA damage was significantly different among combinative exposure of RF signals and APC at 0.2 microg/ml ($p < 0.05$) and at 2 microg/ml ($p < 0.02$). APC at 2 microg/ml concentration also showed significant damage levels ($p < 0.05$) when compared to sham exposed. DNA repair efficiency also varied in a significant way in combinative exposure sets ($p < 0.05$). From these results, it appears that the repair inhibitor APC enhances DNA breaks at 2 microg/ml concentration and that the damage is possibly repairable. Thus, it can be inferred that the in vitro exposure to RF signals induces reversible DNA damage in synergism with APC.

(E) Tkalec M, Stambuk A, Srut M, Malarić K, Klobučar GI. Oxidative and genotoxic effects of 900MHz electromagnetic fields in the earthworm Eisenia fetida. Ecotoxicol Environ Saf. 90:7-12, 2013. (GT, OX, WS)

Accumulating evidence suggests that exposure to radiofrequency electromagnetic field (RF-EMF) can have various biological effects. In this study the oxidative and genotoxic effects were investigated in earthworms Eisenia fetida exposed in vivo to RF-EMF at the mobile phone frequency (900MHz). Earthworms were exposed to the homogeneous RF-EMF at field levels of 10, 23, 41 and 120Vm(-1) for a period of 2h using a Gigahertz Transversal Electromagnetic (GTEM) cell. At the field level of 23Vm(-1) the effect of longer exposure (4h) and field modulation (80% AM 1kHz sinusoidal) was investigated as well. All exposure treatments induced significant genotoxic effect in earthworms coelomocytes detected by the Comet assay, demonstrating DNA damaging capacity of 900MHz electromagnetic radiation. Field modulation additionally increased the genotoxic effect. Moreover, our results indicated the induction of antioxidant stress response in terms of enhanced catalase and glutathione reductase activity as a result of the RF-EMF exposure, and demonstrated the generation of lipid and protein oxidative damage. Antioxidant responses and the potential of RF-EMF to induce damage to lipids, proteins and DNA differed depending on the field level applied, modulation of the field and duration of E. fetida exposure to 900MHz electromagnetic radiation. Nature of detected DNA lesions and oxidative stress as the mechanism of action for the induction of DNA damage are discussed.

(E) Tomruk A, Guler G, Dincel AS. The influence of 1800 MHz GSM-like signals on hepatic oxidative DNA and lipid damage in nonpregnant, pregnant, and newly born rabbits. Cell Biochem Biophys 56:39-47, 2010. (GT, OX, DE, LE)

The aim of our study is to evaluate the possible biological effects of whole-body 1800 MHz GSM-like radiofrequency (RF) radiation exposure on liver oxidative DNA damage and lipid peroxidation levels in nonpregnant, pregnant New Zealand White rabbits, and in their newly borns. Eighteen nonpregnant and pregnant rabbits were used and randomly divided into four groups which were composed of nine rabbits: (i) Group I (nonpregnant control), (ii) Group II (nonpregnant-RF exposed), (iii) Group III (pregnant control), (iv) Group IV (pregnant-RF exposed). Newborns of the pregnant rabbits were also divided into two groups: (v) Group V (newborns of Group III) and (vi) Group VI (newborns of Group III). 1800 MHz GSM-like RF radiation whole-body exposure (15 min/day for a week) was applied to Group II and Group IV. No significant differences were found in liver 8 OHdG/10 dG levels of exposure groups (Group II and Group IV) compared to controls (Group I and Group III). However, in Group II and Group IV malondialdehyde (MDA) and ferrous oxidation in xylenol orange (FOX) levels were increased compared to Group I (P < 0.05, Mann-Whitney). No significant differences were found in liver tissue of 8 OHdG/10 dG and MDA levels between Group VI and Group V (P > 0.05, Mann-Whitney) while liver FOX levels were found significantly increased in Group VI with respect to Group V (P < 0.05, Mann-Whitney). Consequently, the whole-body 1800 MHz GSM-like RF radiation exposure may lead to oxidative destruction as being indicators of subsequent reactions that occur to form oxygen toxicity in tissues.

(E) Trivino Pardo JC, Grimaldi S, Taranta M, Naldi I, Cinti C. Microwave electromagnetic field regulates gene expression in T-lymphoblastoid leukemia CCRF-CEM cell line exposed to 900 MHz. Electromagn Biol Med. 31(1):1-18, 2012. (GE)

Electric, magnetic, and electromagnetic fields are ubiquitous in our society, and concerns have been expressed regarding possible adverse effects of these exposures. Research on Extremely Low-Frequency (ELF) magnetic fields has been performed for more than two decades, and the methodology and quality of studies have improved over time. Studies have consistently shown increased risk for childhood leukemia associated with ELF magnetic fields. There are still inadequate data for other outcomes. More recently, focus has shifted toward Radio Frequencies (RF) exposures from mobile telephony. There are no persuasive data suggesting a health risk, but this research field is still immature with regard to the quantity and quality of available data. This technology is constantly changing and there is a need for continued research on this issue. To investigate whether exposure to high-frequency electromagnetic fields (EMF) could induce adverse health effects, we cultured acute T-lymphoblastoid leukemia cells (CCRF-CEM) in the presence of 900 MHz MW-EMF generated by a transverse electromagnetic (TEM) cell at short and long exposure times. We evaluated the effect of high-frequency EMF on gene expression and we identified functional pathways influenced by 900 MHz MW-EMF exposure.

(E) Trosić I, Pavicić I, Milković-Kraus S, Mladinić M, Zeljezić D. Effect of electromagnetic radiofrequency radiation on the rats' brain, liver and kidney cells measured by comet assay. Coll Antropol 35:1259-1264, 2011. (GT)

The goal of study was to evaluate DNA damage in rat's renal, liver and brain cells after in vivo exposure to radiofrequency/microwave (Rf/Mw) radiation of cellular phone frequencies range. To determine DNA damage, a single cell gel electrophoresis/comet assay was used. Wistar rats (male, 12 week old, approximate body weight 350 g) (N = 9) were exposed to the carrier frequency of 915 MHz with Global System Mobile signal modulation (GSM), power density of 2.4 W/m2, whole body average specific absorption rate SAR of 0.6 W/kg. The animals were irradiated for one hour/day, seven days/week during two weeks period. The exposure set-up was Gigahertz Transversal Electromagnetic Mode Cell (GTEM--cell). Sham irradiated controls (N = 9) were apart of the study. The body temperature was measured before and after exposure. There were no differences in temperature in between control and treated animals. Comet assay parameters such as the tail length and tail intensity were evaluated. In comparison with tail length in controls (13.5 +/- 0.7 microm), the tail was slightly elongated in brain cells of irradiated animals (14.0 +/- 0.3 microm). The tail length obtained for liver (14.5 +/- 0.3 microm) and kidney (13.9 +/- 0.5 microm) homogenates notably differs in comparison with matched sham controls (13.6 +/- 0.3 microm) and (12.9 +/- 0.9 microm). Differences in tail intensity between control and exposed animals were not significant. The results of this study suggest that, under the experimental conditions applied, repeated 915 MHz irradiation could be a cause of DNA breaks in renal and liver cells, but not affect the cell genome at the higher extent compared to the basal damage.

(NE) Valbonesi P, Franzellitti S, Piano A, Contin A, Biondi C, Fabbri E. Evaluation of HSP70 Expression and DNA damage in cells of a human trophoblast cell line exposed to 1.8 GHz amplitude-modulated radiofrequency fields. Radiat Res 169:270-279, 2008. (GT, GE)

The aim of this study was to determine whether high-frequency electromagnetic fields (EMFs) could induce cellular effects. The human trophoblast cell line HTR-8/SVneo was used as a model to evaluate the expression of proteins (HSP70 and HSC70) and genes (HSP70A, B, C and HSC70) of the HSP70 family and the primary DNA damage response after nonthermal exposure to pulse-modulated 1817 MHz sinusoidal waves (GSM-217 Hz; 1 h; SAR of 2 W/kg). HSP70 expression was significantly enhanced by heat, which was applied as the prototypical stimulus. The HSP70A, B and C transcripts were differentially expressed under basal conditions, and they were all significantly induced above basal levels by thermal stress. Conversely, HSC70 protein and gene expression was not influenced by heat. Exposing HTR-8/SVneo cells to high-frequency EMFs did not change either HSP70 or HSC70 protein or gene expression. A significant increase in DNA strand breaks was caused by exposure to HO, which was used as a positive stimulus; however, no effect was observed after exposure of cells to high-frequency EMFs. Overall, no evidence was found that a 1-h exposure to GSM-217 Hz induced a HSP70-mediated stress response or primary DNA damage in HTR-8/SVneo cells. Nevertheless, further investigations on trophoblast cell responses after exposure to GSM signals of different types and durations are needed.

(E) Valbonesi P, Franzellitti S, Bersani F, Contin A, Fabbri E. Effects of the exposure to intermittent 1.8 GHz radio frequency electromagnetic fields on HSP70 expression and MAPK signaling pathways in PC12 cells. Int J Radiat Biol. 2014 Feb 11. [Epub ahead of print] (GE, WS)

Purpose: We previously reported effects on heat shock protein 70 (HSP70) mRNA expression, a cytoprotective protein induced under stressful condition, in human trophoblast cells exposed to amplitude-modulated Global System for Mobile Communication (GSM) signals. In the present work the same experimental conditions were applied to the rat PC12 cells, in order to assess the stress responses mediated by HSP70 and by the Mitogen Activated Protein Kinases (MAPK) in neuronal-like cells, an interesting model to study possible effects of mobile phone frequencies exposure. Materials and methods: HSP70 gene expression level was evaluated by reverse transcriptase polymerase chain reaction, HSP70 protein expression and MAPK phosphorylation were assessed by Western blotting. PC12 cells were exposed for 4, 16 or 24 h to 1.8 GHz continuous wave signal (CW, carrier frequency without modulation) or to two different GSM modulation schemes, GSM-217Hz and GSM-Talk (which generates temporal changes between two different GSM signals, active during talking or listening phases respectively, thus simulating a typical conversation). Specific adsorption rate (SAR) was 2 W/kg. Results: After PC12 cells exposure to the GSM-217Hz signal for 16 or 24 h, HSP70 transcription significantly increased, whereas no effect was observed in cells exposed to the CW or GSM-Talk signals. HSP70 protein expression and three different MAPK signaling pathways were not affected by the exposure to any of the three different 1.8 GHz signals. Conclusion: The positive effect on HSP70 mRNA expression, observed only in cells exposed to the GSM-217Hz signal, is a repeatable response previously reported in human trophoblast cells and now confirmed in PC12 cells. Further investigations towards a possible role of 1.8 GHz signal modulation are therefore advisable.

(NE) Verschaeve L, Heikkinen P, Verheyen G, Van Gorp U, Boonen F, Vander Plaetse F, Maes A, Kumlin T, Maki-Paakkanen J, Puranen L, Juutilainen J. Investigation of co-genotoxic effects of radiofrequency electromagnetic fields in vivo. Radiat Res 165:598-607, 2006. (GT, LE, IA)

We investigated the possible combined genotoxic effects of radiofrequency (RF) electromagnetic fields (900 MHz, amplitude modulated at 217 Hz, mobile phone signal) with the drinking water mutagen and carcinogen 3-chloro-4-(dichloromethyl)-5-hydroxy-2(5H)-furanone (MX). Female rats were exposed to RF fields for a period of 2 years for 2 h per day, 5 days per week at average whole-body specific absorption rates of 0.3 or 0.9 W/kg. MX was given in the drinking water at a concentration of 19 mug/ml. Blood samples were taken at 3, 6 and 24 months of exposure and brain and liver samples were taken at the end of the study (24 months). DNA damage was assessed in all samples using the alkaline comet assay, and micronuclei were determined in erythrocytes. We did not find significant genotoxic activity of MX in blood and liver cells. However, MX induced DNA damage in rat brain. Co-exposures to MX and RF radiation did not significantly increase the response of blood, liver and brain cells compared to MX exposure only. In conclusion, this 2-year animal study involving long-term exposures to RF radiation and MX did not provide any evidence for enhanced genotoxicity in rats exposed to RF radiation.

(NE) Vijayalaxmi. Cytogenetic studies in human blood lymphocytes exposed in vitro to 2.45 GHz or 8.2 GHz radiofrequency radiation. Radiat Res 166, 532–538, 2006. (GT)

Peripheral blood samples collected from healthy human volunteers were exposed in vitro to 2.45 GHz or 8.2 GHz pulsed-wave radiofrequency (RF) radiation. The net forward power, average power density, mean specific absorption rate, and the temperature maintained during the 2-h exposure of the cells to 2.45 GHz or 8.2 GHz were, respectively, 21 W or 60 W, 5 mW/cm2 or 10 mW/cm2, 2.13 W/kg or 20.71 W/kg, and 36.9 ± 0.1°C or 37.5 ± 0.2°C. Aliquots of the same blood samples that were either sham-exposed or exposed in vitro to an acute dose of 1.5 Gy γ radiation were used as unexposed and positive controls, respectively. Cultured lymphocytes were examined to determine the extent of cytogenetic damage assessed from the incidence of chromosomal aberrations and micronuclei. Under the conditions used to perform the experiments, the levels of damage in RF-radiation-exposed and sham-exposed lymphocytes were not significantly different. Also, there were no significant differences in the response of unstimulated lymphocytes and lymphocytes stimulated with phytohemagglutinin when exposed to 8.2 GHz RF radiation. In contrast, the positive control cells that had been subjected to γ irradiation exhibited significantly more damage than RF-radiation- and sham-exposed lymphocytes.

(NE) Waldmann P, Bohnenberger S, Greinert R, Hermann-Then B, Heselich A, Klug SJ, Koenig J, Kuhr K, Kuster N, Merker M, Murbach M, Pollet D, Schadenboeck W, Scheidemann-Wesp U, Schwab B, Volkmer B, Weyer V, Blettner M. Influence of GSM Signals on Human Peripheral Lymphocytes: Study of Genotoxicity. Radiat Res. 2013 Jan 14. [Epub ahead of print] (GT)

Exposure to radiofrequency (RF) electromagnetic fields (EMF) is continuously increasing worldwide. Yet, conflicting results of a possible genotoxic effect of RF EMF continue to be discussed. In the present study, a possible genotoxic effect of RF EMF (GSM, 1,800 MHz) in human lymphocytes was investigated by a collaboration of six independent institutes (institutes a, b, c, d, e, h). Peripheral blood of 20 healthy, nonsmoking volunteers of two age groups (10 volunteers 16-20 years old and 10 volunteers 50-65 years old) was taken, stimulated and intermittently exposed to three specific absorption rates (SARs) of RF EMF (0.2 W/kg, 2 W/kg, 10 W/kg) and sham for 28 h (institute a). The exposures were performed in a setup with strictly controlled conditions of temperature and dose, and randomly and automatically determined waveguide SARs, which were designed and periodically maintained by ITIS (institute h). Four genotoxicity tests with different end points were conducted (institute a): chromosome aberration test (five types of structural aberrations), micronucleus test, sister chromatid exchange test and the alkaline comet assay (Olive tail moment and % DNA). To demonstrate the validity of the study, positive controls were implemented. The genotoxicity end points were evaluated independently by three laboratories blind to SAR information (institute c = laboratory 1; institute d = laboratory 2; institute e = laboratory 3). Statistical analysis was carried out by institute b. Methods of primary statistical analysis and rules to adjust for multiple testing were specified in a statistical analysis plan based on a data review before unblinding. A linear trend test based on a linear mixed model was used for outcomes of comet assay and exact permutation test for linear trend for all other outcomes. It was ascertained that only outcomes with a significant SAR trend found by at least two of three analyzing laboratories indicated a substantiated suspicion of an exposure effect. On the basis of these specifications, none of the nine end points tested for SAR trend showed a significant and reproducible exposure effect. Highly significant differences between sham exposures and positive controls were detected by each analyzing laboratory, thus validating the study. In conclusion, the results show no evidence of a genotoxic effect induced by RF EMF (GSM, 1,800 MHz).

(E) Wu W, Yao K, Wang KJ, Lu DQ, He JL, Xu LH, Sun WJ. [Blocking 1800 MHz mobile phone radiation-induced reactive oxygen species production and DNA damage in lens epithelial cells by noise magnetic fields.]Zhejiang Da XueXueBao Yi Xue Ban 37:34-38, 2008. [Article in Chinese] (GT, IA, OX)

OBJECTIVE: To investigate whether the exposure to the electromagnetic noise can block reactive oxygen species (ROS) production and DNA damage of lens epithelial cells induced by 1800 MHz mobile phone radiation. METHODS: The DCFH-DA method and comet assay were used respectively to detect the intracellular ROS and DNA damage of cultured human lens epithelial cells induced by 4 W/kg 1800 MHz mobile phone radiation or/and 2microT electromagnetic noise for 24 h intermittently. RESULT: 1800 MHz mobile phone radiation at 4 W/kg for 24 h increased intracellular ROS and DNA damage significantly (P<0.05). However, the ROS level and DNA damage of mobile phone radiation plus noise group were not significant enhanced (P>0.05) as compared to sham exposure group. Conclusion: Electromagnetic noise can block intracellular ROS production and DNA damage of human lens epithelial cells induced by 1800 MHz mobile phone radiation.

(E) Xu S, Zhong M, Zhang L, Zhou Z, Zhang W, Wang Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Yu Z. Exposure to 1800 MHz radiofrequency radiation induces

oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res 1311:189-196. 2010. (GT, OX)

Increasing evidence indicates that oxidative stress may be involved in the adverse effects of radiofrequency (RF) radiation on the brain. Because mitochondrial DNA (mtDNA) defects are closely associated with various nervous system diseases and mtDNA is highly susceptible to oxidative stress, the purpose of this study was to determine whether radiofrequency radiation can cause oxidative damage to mtDNA. In this study, we exposed primary cultured cortical neurons to pulsed RF electromagnetic fields at a frequency of 1800 MHz modulated by 217 Hz at an average special absorption rate (SAR) of 2 W/kg. At 24h after exposure, we found that RF radiation induced a significant increase in the levels of 8-hydroxyguanine (8-OHdG), a common biomarker of DNA oxidative damage, in the mitochondria of neurons. Consistent with this finding, the copy number of mtDNA and the levels of mitochondrial RNA (mtRNA) transcripts showed an obvious reduction after RF exposure. Each of these mtDNA disturbances could be reversed by pretreatment with melatonin, which is known to be an efficient in the brain. Together, these results suggested that 1800 MHz RF radiation could cause oxidative damage to mtDNA in primary cultured neurons. Oxidative damage to mtDNA may account for the neurotoxicity of RF radiation in the brain.

(E) Xu S, Chen G, Chen C, Sun C, Zhang D, Murbach M, Kuster N, Zeng Q, Xu Z. Cell Type-Dependent Induction of DNA Damage by 1800 MHz Radiofrequency Electromagnetic Fields Does Not Result in Significant Cellular Dysfunctions. PLoS One. 8(1):e54906, 2013. (GT, CS)

BACKGROUND: Although IARC clarifies radiofrequency electromagnetic fields (RF-EMF) as possible human carcinogen, the debate on its health impact continues due to the inconsistent results. Genotoxic effect has been considered as a golden standard to determine if an environmental factor is a carcinogen, but the currently available data for RF-EMF remain controversial. As an environmental stimulus, the effect of RF-EMF on cellular DNA may be subtle. Therefore, more sensitive method and systematic research strategy are warranted to evaluate its genotoxicity. **OBJECTIVES:** To determine whether RF-EMF does induce DNA damage and if the effect is cell-type dependent by adopting a more sensitive method γH2AX foci formation; and to investigate the biological consequences if RF-EMF does increase γH2AX foci formation. **METHODS:** Six different types of cells were intermittently exposed to GSM 1800 MHz RF-EMF at a specific absorption rate of 3.0 W/kg for 1 h or 24 h, then subjected to immunostaining with anti-γH2AX antibody. The biological consequences in γH2AX-elevated cell type were further explored with comet and TUNEL assays, flow cytometry, and cell growth assay. **RESULTS:** Exposure to RF-EMF for 24 h significantly induced γH2AX foci formation in Chinese hamster lung cells and Human skin fibroblasts (HSFs), but not the other cells. However, RF-EMF-elevated γH2AX foci formation in HSF cells did not result in detectable DNA fragmentation, sustainable cell cycle arrest, cell proliferation or viability change. RF-EMF exposure slightly but not significantly increased the cellular ROS level. **CONCLUSIONS:** RF-EMF induces DNA damage in a cell type-dependent manner, but the elevated γH2AX foci formation in HSF cells does not result in significant cellular dysfunctions.

(NE) Yadav AS, Sharma MK. Increased frequency of micronucleated exfoliated cells among humans exposed in vivo to mobile telephone radiations. Mutat Res.650(2):175-180, 2008. (LE, GT, HU)

The health concerns have been raised following the enormous increase in the use of wireless mobile telephones throughout the world. This investigation had been taken, with the motive to find out whether mobile phone radiations cause any in vivo effects on the frequency of micronucleated exfoliated cells in the exposed subjects. A total of 109 subjects including 85 regular mobile phone users (exposed) and 24 non-users (controls) had participated in this study. Exfoliated cells were obtained by swabbing the buccal-mucosa from exposed as well as sex-age-matched controls. One thousand exfoliated cells were screened from each individual for nuclear anomalies including micronuclei (MN), karyolysis (KL), karyorrhexis (KH), broken egg (BE) and binucleated (BN) cells. The average daily duration of exposure to mobile phone radiations is 61.26 min with an overall average duration of exposure in term of years is 2.35 years in exposed subjects along with the 9.84+/-0.745 micronucleated cells (MNCs) and 10.72+/-0.889 total micronuclei (TMN) as compared to zero duration of exposure along with average 3.75+/-0.774 MNC and 4.00+/-0.808 TMN in controls. The means are significantly different in case of MNC and TMN at 0.01% level of significance. The mean of KL in controls is 13.17+/-2.750 and in exposed subjects is 13.06+/-1.793. The value of means of KH in exposed subjects (1.84+/-0.432) is slightly higher than in controls (1.42+/-0.737). Mean frequency of broken egg is found to be more in exposed subjects (0.65+/-0.276) as compared to controls (0.50+/-0.217). Frequency of presence of more than one nucleus in a cell (binucleated) is also higher in exposed (2.72+/-0.374) in comparison to controls (0.67+/-0.231). Although there is a slight increase in mean frequency of KH, BE and BN in exposed subjects but the difference is not found statistically significant. Correlation between 0-1, 1-2, 2-3 and 3-4 years of exposure and the frequency of MNC and TMN has been calculated and found to be positively correlated.

(E) Yan JG, Agresti M, Zhang LL, Yan Y, Matloub HS. Upregulation of specific mRNA levels in rat brain after cell phone exposure. Electromagn Biol Med. 27(2):147-154, 2008. (LE, GE)

Adult Sprague-Dawley rats were exposed to regular cell phones for 6 h per day for 126 days (18 weeks). RT-PCR was used to investigate the changes in levels of mRNA synthesis of several injury-associated proteins. Calcium ATPase, Neural Cell Adhesion Molecule, Neural Growth Factor, and Vascular Endothelial Growth Factor were evaluated. The results showed statistically significant mRNA up-regulation of these proteins in the brains of rats exposed to cell phone radiation. These results indicate that relative chronic exposure to cell phone microwave radiation may result in cumulative injuries that could eventually lead to clinically significant neurological damage.

(E) Yao K, Wu W, Wang K, Ni S, Ye P, Yu Y, Ye J, Sun L. Electromagnetic noise inhibits radiofrequency radiation-induced DNA damage and reactive oxygen species increase in human lens epithelial cells. Mol Vis 14:964-969, 2008. (GT, IA, OX)

PURPOSE: The goal of this study was to investigate whether superposing of electromagnetic noise could block or attenuate DNA damage and intracellular reactive oxygen species (ROS) increase of cultured human lens epithelial cells (HLECs) induced by acute exposure to 1.8 GHz radiofrequency field (RF) of the Global System for Mobile Communications (GSM). METHODS: An sXc-1800 RF exposure system was used to produce a GSM signal at 1.8 GHz (217 Hz amplitude-modulated) with the specific absorption rate (SAR) of 1, 2, 3, and 4 W/kg. After 2 h of intermittent exposure, the ROS level was assessed by the fluorescent probe, 2',7'-dichlorodihydrofluorescein diacetate (DCFH-DA). DNA damage to HLECs was examined by alkaline comet assay and the phosphorylated form of histone variant H2AX (gammaH2AX) foci formation assay. RESULTS: After exposure to 1.8 GHz RF for 2 h, HLECs exhibited significant intracellular ROS increase in the 2, 3, and 4 W/kg groups. RF radiation at the SAR of 3 W/kg and 4 W/kg could induce significant DNA damage, examined by alkaline comet assay, which was used to detect mainly single strand breaks (SSBs), while no statistical difference in double strand breaks (DSBs), evaluated by gammaH2AX foci, was found between RF exposure (SAR: 3 and 4 W/kg) and sham exposure groups. When RF was superposed with 2 muT electromagnetic noise could block RF-induced ROS increase and DNA damage. CONCLUSIONS: DNA damage induced by 1.8 GHz radiofrequency field for 2 h, which was mainly SSBs, may be associated with the increased ROS production. Electromagnetic noise could block RF-induced ROS formation and DNA damage.

(NE) Yildirim MS, Yildirim A, Zamani AG, Okudan N. Effect of mobile phone station on micronucleus frequency and chromosomal aberrations in human blood cells. Genet Couns. 21(2):243-251, 2010. (HU, LE, GT)

The use of mobile telephones has rapidly increased worldwide as well as the number of mobile phone base stations that lead to rise low level radiofrequency emissions which may in turn have possible harm for human health. The national radiation protection board has published the known effects of radio waves exposure on humans living close to mobile phone base stations. However, several studies have claimed that the base station has detrimental effects on different tissues. In this study, we aimed to evaluate the effects of mobile phone base stations on the micronucleus (MN) frequency and chromosomal aberrations on blood in people who were living around mobile phone base stations and healthy controls. Frequency of MN and chromosomal aberrations in study and control groups was 8.96 +/- 3.51 and 6.97 +/- 1.52 (p: 0.16); 0.36 +/- 0.31 and 0.75 +/- 0.61 (p: 0.07), respectively. Our results show that there was not a significant difference of MN frequency and chromosomal aberrations between the two study groups. The results claim that cellular phones and their base stations do not produce important carcinogenic changes.

(E) Zalata, A., A. Z. El-Samanoudy, D. Shaalan, Y. El-Baiomy, and T. Mostafa. In vitro effect of cell phone radiation on motility, DNA fragmentation and clusterin gene expression of sperm. Int J Fertil Steril, In Press. Published online ahead of print. (GT, GE, RP)

Background: Use of cellular phones that emits radiofrequency electromagnetic field (RF-EMF) has been increased exponentially and became a part of everyday life. This study aimed to investigate the effects of RF-EMF radiation emitted from cellular phones on sperm motility variables, sperm DNA fragmentation and clusterin (CLU) gene expression. Materials and Methods: 124 semen samples were grouped into; normozoospermia (N, n=26), asthenozoospermia (A, n=32), asthenoteratozoospermia (AT, n=31) and oligoasthenoteratozoospermia (OAT, n=35). Semen samples were divided into two aliquots; samples not exposed to cell phone and samples exposed to cell phone radiation (850 MHz, maximum power < 1 watt; SAR 1.46 W/kg at 10 cm distance) for 1 hr. Before and immediately

after exposure both aliquots were subjected to assessment of sperm motility, acrosin activity, sperm DNA fragmentation and CLU gene expression. Statistical differences were analyzed using paired t-student test for comparisons where P< 0.05 was set as significant. Results: There was significant decrease in sperm motility, sperm linear velocity, sperm linearity index, sperm acrosin activity and significant increase in sperm DNA fragmentation percent, CLU gene expression and CLU protein levels in the exposed semen samples to RF-EMF compared with non- exposed samples in OAT > AT > A > N groups (P<0.05).
Conclusions: Cell phone emissions have a negative impact on exposed sperm motility indices, sperm acrosin activity, sperm DNA fragmentation and CLU gene expression especially in OAT cases.

(NE) Zeni O, Schiavoni A, Perrotta A, Forigo D, Deplano M, Scarfi MR. Evaluation of genotoxic effects in human leukocytes after in vitro exposure to 1950 MHz UMTS radiofrequency field. Bioelectromagnetics 29:177-184, 2008. (GT)

In the present study the third generation wireless technology of the Universal Mobile Telecommunication System (UMTS) signal was investigated for the induction of genotoxic effects in human leukocytes. Peripheral blood from six healthy donors was used and, for each donor, intermittent exposures (6 min RF on, 2 h RF off) at the frequency of 1950 MHz were conducted at a specific absorption rate of 2.2 W/kg. The exposures were performed in a transverse electro magnetic (TEM) cell hosted in an incubator under strictly controlled conditions of temperature and dosimetry. Following long duration intermittent RF exposures (from 24 to 68 h) in different stages of the cell cycle, micronucleus formation was evaluated by applying the cytokinesis block micronucleus assay, which also provides information on cell division kinetics. Primary DNA damage (strand breaks/alkali labile sites) was also investigated following 24 h of intermittent RF exposures, by applying the alkaline single cell gel electrophoresis (SCG)/comet assay. Positive controls were included by treating cell cultures with Mitomycin-C and methylmethanesulfonate for micronucleus and comet assays, respectively. The results obtained indicate that intermittent exposures of human lymphocytes in different stages of cell cycle do not induce either an increase in micronucleated cells, or change in cell cycle kinetics; moreover, 24 h intermittent exposures also fail to affect DNA structure of human leukocytes soon after the exposures, likely indicating that repairable DNA damage was not induced.

(E) Zhang DY, Xu ZP, Chiang H, Lu DQ, Zeng QL. [Effects of GSM 1800 MHz radiofrequency electromagnetic fields on DNA damage in Chinese hamster lung cells.] Zhonghua Yu Fang Yi XueZaZhi 40:149-152, 2006. [Article in Chinese] (GT)

OBJECTIVE: To study the effects of GSM 1800 MHz radiofrequency electromagnetic fields (RF EMF) on DNA damage in Chinese hamster lung (CHL) cells. METHODS: The cells were intermittently exposed or sham-exposed to GSM 1800 MHz RF EMF (5 minutes on/10 minutes off) at a special absorption rate (SAR) of 3.0 W/kg for 1 hour or 24 hours. Meanwhile, cells exposed to 2-acetaminofluorene, a DNA damage agent, at a final concentration of 20 mg/L for 2 hours were used as positive control. After exposure, cells were fixed by using 4% paraformaldehyde and processed for phosphorylated form of H2AX (gammaH2AX) immunofluorescence measurement. The primary antibody used for immunofluorescence was mouse monoclonal antibody against gammaH2AX and the secondary antibody was fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG. Nuclei were counterstained with 4, 6-diamidino-2-phenylindole (DAPI). The gammaH2AX foci and nuclei were visualized with an Olympus AX70 fluorescent microscope. Image Pro-Plus software was used to count the gammaH2AX foci in each cell. For each exposure condition, at least 50 cells were selected to detect gammaH2AX foci. Cells were classified as positive when more than five foci were detected. The percentage of gammaH2AX foci positive cells was adopted as the index of DNA damage. RESULTS: The percentage of gammaH2AX foci positive cell of 1800 MHz RF EMF exposure for 24 hours (37.9 +/- 8.6)% or 2-acetylaminofluorene exposure (50.9 +/- 9.4)% was significantly higher compared with the sham-exposure (28.0 +/- 8.4)%. However, there was no significant difference between the sham-exposure and RF EMF exposure for 1 hour (31.8 +/- 8.7)%. CONCLUSION: 1800 MHz RF EMF (SAR, 3.0 W/kg) for 24 hours might induce DNA damage in CHL cells.

(E) Zhang SZ, Yao GD, Lu DQ, Chiang H, Xu ZP. [Effect of 1.8 GHz radiofrequency electromagnetic fields on gene expression of rat neurons]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 26(8):449-452, 2008. [Article in Chinese] (GE, WS)

OBJECTIVE: To investigate the changes of gene expression in rat neuron induced by 1.8 GHz radiofrequency electromagnetic fields (RF EMF) to screen for RF EMF-responsive genes and the effect of different exposure times and modes on the gene expression in neuron. METHODS: Total RNA was extracted immediately and purified from the primary culture of neurons after intermittent exposed or sham-exposed to a frequency of 1.8 GHz RF EMF for 24 hours at an average special absorption rate (SAR) of 2 W/kg. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron. Differentially expressed genes (Egr-1, Mbp and Plp) were further confirmed by semi-quantitative revere transcription polymerase chain reaction (RT PCR). The expression levels of Egr-1, Mbp and Plp were observed at different exposure times (6, 24 h) and modes (intermittent and continuous exposure). RESULTS: Among 1200 candidate genes, 24 up-regulated and 10 down-regulated genes were found by using Affymetrix microarray suite software 5.0 which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. Under 24 h and 6 h intermittent exposure, Egr-1 and Plp in experiment groups showed statistic significance ($P < 0.05$) compared with the control groups, while expression of Mbp did not change significantly ($P > 0.05$). After 24 h continuous exposure, Egr-1 and Mbp in experiment groups showed statistic significance ($P < 0.05$) compared with the control group, while expression of Plp did not change significantly ($P > 0.05$). Under the same exposure mode 6 h, expression of all the 3 genes did not change significantly. Different times (6, 24 h) and modes (intermittent and continuous exposure) of exposure exerted remarkable different influences on the expression of Egr-1, Mbp, Plp genes ($P < 0.01$). CONCLUSION: The changes of many genes transcription were involved in the effect of 1.8 GHz RF EMF on rat neurons; Down-regulation of Egr-1 and up-regulation of Mbp, Plp indicated the negative effects of RF EMF on neurons; The effect of RF intermittent exposure on gene expression was more obvious than that of continuous exposure; The effect of 24 h RF exposure (both intermittent and continuous) on gene expression was more obvious than that of 6 h (both intermittent and continuous).

(E) Zhao R, Zhang S, Xu Z, Ju L, Lu D, Yao G. Studying gene expression profile of rat neuron exposed to 1800MHz radiofrequency electromagnetic fields with cDNA microassay. Toxicology 235:167-175, 2007. (GE)

A widespread use of mobile phone (MP) evokes a growing concern for their possible adverse effects on human, especially the brain. Gene expression is a unique way of characterizing how cells and organism adapt to changes in the external environment, so the aim of this investigation was to determine whether 1800 MHz radiofrequency electromagnetic fields (RF EMF) can influence the gene expression of neuron. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron after exposed to the pulsed RF EMF at a frequency of 1800 MHz modulated by 217 Hz which is commonly used in MP. Among 1200 candidate genes, 24 up-regulated genes and 10 down-regulated genes were identified after 24-h intermittent exposure at an average special absorption rate (SAR) of 2 W/kg, which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. The results were further confirmed by quantitative real-time polymerase chain reaction (RT PCR). The present results indicated that the gene expression of rat neuron could be altered by exposure to RF EMF under our experimental conditions.

(E) Zhao TY, Zou SP, Knapp PE. Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. Neurosci Lett. 412(1):34-38, 2007. (GE, CS)

The health effects of cell phone radiation exposure are a growing public concern. This study investigated whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to a working Global System for Mobile Communication (GSM) cell phone rated at a frequency of 1900MHz. Primary cultures were exposed to cell phone emissions for 2h. We used array analysis and real-time RT-PCR to show up-regulation of caspase-2, caspase-6 and Asc (apoptosis associated speck-like protein containing a card) gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons, but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The effects are specific since up-regulation was not seen for other genes associated with apoptosis, such as caspase-9 in either neurons or astrocytes, or Bax in neurons. The results show that even relatively short-term exposure to cell phone radiofrequency emissions can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

(E) Zhijian C, Xiaoxue L, Yezhen L, Shijie C, Lifen J, Jianlin L, Deqiang L, Jiliang H. Impact of 1.8-GHz radiofrequency radiation (RFR) on DNA damage and repair induced by doxorubicin in human B-cell lymphoblastoid cells. Mutat Res. 695(1-2):16-21, 2010. (GT, IA)

In the present in vitro study, a comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR, SAR of 2W/kg) can influence DNA repair in human B-cell lymphoblastoid cells exposed to doxorubicin (DOX) at the doses of 0microg/ml, 0.05microg/ml, 0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml. The combinative exposures to RFR with DOX were divided into five categories. DNA damage was detected at 0h, 6h, 12h, 18h and 24h after exposure to DOX via the comet assay, and the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage. The results demonstrated that (1) RFR could not directly induce DNA damage of human B-cell lymphoblastoid cells; (2) DOX could significantly induce DNA damage of human B-cell lymphoblastoid cells with the dose-effect relationship, and there were special repair characteristics of DNA damage induced by DOX; (3) E-E-E type (exposure to RFR for 2h, then simultaneous exposure to RFR and DOX, and exposure to RFR for 6h, 12h, 18h and 24h after exposure to DOX) combinative exposure could obviously influence DNA repair at 6h and 12h after exposure to DOX for four DOX doses (0.075microg/ml, 0.10microg/ml, 0.15microg/ml and 0.20microg/ml) in human B-cell lymphoblastoid cells.

(NE) Zhijian C, Xiaoxue L, Yezhen L, Deqiang L, Shijie C, Lifen J, Jianlin L, Jiliang H. Influence of 1.8-GHz (GSM) radiofrequency radiation (RFR) on DNA damage and repair induced by X-rays in human leukocytes in vitro. Mutat Res. 677(1-2):100-104, 2009. (GT, IA)

In the present study, the in vitro comet assay was used to determine whether 1.8-GHz radiofrequency radiation (RFR) can influence DNA repair in human leukocytes exposed to X-rays. The specific energy absorption rate (SAR) of 2 W/kg (the current European safety limit) was applied. The leukocytes from four young healthy donors were intermittently exposed to RFR for 24 h (fields on for 5 min, fields off for 10 min), and then irradiated with X-rays at doses of 0.25, 0.5, 1.0 and 2.0 Gy. DNA damage to human leukocytes was detected using the comet assay at 0, 15, 45, 90, 150 and 240 min after exposure to X-rays. Using the comet assay, the percent of DNA in the tail (% tail DNA) served as the indicator of DNA damage; the DNA repair percentage (DRP) served as the indicator of the DNA repair speed. The results demonstrated that (1) the DNA repair speeds of human leukocytes after X-ray exposure exhibited individual differences among the four donors; (2) the intermittent exposures of 1.8-GHz RFR at the SAR of 2 W/kg for 24 h did not directly induce DNA damage or exhibit synergistic effects with X-rays on human leukocytes.

(NE) Ziemann C, Brockmeyer H, Reddy SB, Vijayalaxmi, Prihoda TJ, Kuster N, Tillmann T, Dasenbrock C. Absence of genotoxic potential of 902 MHz (GSM) and 1747 MHz (DCS) wireless communication signals: In vivo two-year bioassay in B6C3F1 mice. Int J Radiat Biol. 85(5):454-464, 2009. (GT, LE)

PURPOSE: The aim of the present investigation was to determine the incidence of micronuclei in peripheral blood erythrocytes of B6C3F1 mice that had been chronically exposed to radiofrequencies (RF) used for mobile communication. MATERIALS AND METHODS: 'Ferris wheels' were used to expose tube-restrained male and female mice to simulated environmental RF signals of the Global System for Mobile Communications (GSM, 902 MHz) or Digital Cellular System (DCS, 1747 MHz). RF signals were applied to the mice for 2 hours/day on 5 days/week for two years, at maximal whole-body-averaged specific absorption rates of 0.4, 1.3, and 4.0 W/kg body weight. Concurrent sham-exposed mice, cage controls, and positive controls injected with mitomycin C were included in this investigation. At necropsy, peripheral blood smears were prepared, and coded slides were stained using May-Grunwald-Giemsa or acridine orange. The incidence of micronuclei was recorded for each mouse in 2000 polychromatic and 2000 normochromatic erythrocytes. RESULTS: There were no significant differences in the frequency of micronuclei between RF-exposed, sham-exposed, and cage control mice, irrespective of the staining/counting method used. Micronuclei were, however, significantly increased in polychromatic erythrocytes of the positive control mice.

CONCLUSIONS: In conclusion, the data did not indicate RF-induced genotoxicity in mice after two years of exposure.

APPENDIX B - ABSTRACTS ON GENETIC EFFECTS OF EXTREMELY-LOW FREQUENCY ELECTROMAGNETIC FIELDS (2007-2014)

Below is a key to abbreviations used throughout the following list of abstracts for recent papers published since 2006 and serve as my comments to help the reader quickly identify the significance of each work. The summary sentences by each author are underlined. The list is divided into RF effects papers, and ELF effects papers.

(E- effect observed; NE- no effect observed) (LE- long term exposure; GT- genotoxic effect, e.g., DNA damage, micronucleus formation, chromosome alterations; GE- gene expression; HU- human study; OX- oxidative effects, i.e., involvement of free radicals and oxidative enzymes; IA- interaction with other factors to cause genetic effects; DE- effects on developing animals; RP- reproduction, e.g., sperm damage; EH- compared with electro-hypersensitive subjects; WS- waveform specific effect, e.g., modulation and frequency; CS- cell type specific effect).

(NE) Albert GC, McNamee JP, Marro L, Bellier PV, Prato FS, Thomas AW. Assessment of genetic damage in peripheral blood of human volunteers exposed (whole-body) to a 200 muT, 60 Hz magnetic field. Int J Radiat Biol. 85(2):144-152, 2009. (GT, IA)

AIM: To investigate the extent of damage in nucleated cells in peripheral blood of healthy human volunteers exposed to a whole-body 60 Hz, 200 microT magnetic field. **MATERIALS AND METHODS:** In this study, 10 male and 10 female healthy human volunteers received a 4 h whole-body exposure to a 200 microT, 60 Hz magnetic field. In addition, five males and five females were treated in a similar fashion, but were exposed to sham conditions. For each subject, a blood sample was obtained prior to the exposure period and aliquots were used as negative- (pre-exposure) and positive- [1.5 Gray (Gy) (60)Cobalt ((60)Co) gamma-irradiation] controls. At the end of the 4 h exposure period, a second blood sample was obtained. The extent of DNA damage was assessed in peripheral human blood leukocytes from all samples using the alkaline comet assay. To detect possible clastogenic effects, the incidence of micronuclei was assessed in phytohemagglutinin (PHA)-stimulated lymphocytes using the cytokinesis-block micronucleus assay. **RESULTS:** There was no evidence of either increased DNA damage, as indicated by the alkaline comet assay, or increased incidence of micronuclei (MN) in the magnetic field exposed group. However, an in vitro exposure of 1.5 Gy gamma-irradiation caused a significant increase in both DNA damage and MN induction. **CONCLUSIONS:** This study found no evidence that an acute, whole-body exposure to a 200 microT, 60 Hz magnetic field for 4 hours could cause DNA damage in human blood.

(E) Alcaraz M, Olmos E, Alcaraz-Saura M, Achel DG, Castillo J. Effect of long-term 50 Hz magnetic field exposure on the micronucleated polychromatic erythrocytes of mice. Electromagn Biol Med. 2013 Jun 19. [Epub ahead of print] (GT)

Abstract In recent years extremely low-frequency magnetic fields (ELF-EMF) have become widely used in human activities, leading to an increased chance of exposure to ELF-EMF. There are few reports on in vivo mammalian genotoxic effects using micronucleus (MN) assays, which generally have been used as a short-term screening system. We analyzed the possible genotoxic effect induced by long-term exposure (7, 14, 21, 28 d) of a 50 Hz ELM-MF to mice by measuring the increase in frequency of micronucleated polychromatic erythrocyte in their bone marrow (MNPCEs) and we compared it with that induced by 50 cGy of X-rays. Subsequently, we tried to reduce this chromosomal damage by administering four antioxidants substances with radioprotective capacities: dimethyl sulfoxide (DMSO), 6-n-propyl-2-thiouracil (PTU), grape-procyanidins (P) and citrus flavonoids extract (CE). The increase in micronucleated cells was higher in both physical treatments (Control < ELF-EMF ($p < 0.01$) <X-rays ($p > 0.001$)); however, the antioxidant substances only showed a genoprotective capacity against the damage induced by ionizing radiation (Ci > PTU = DMSO ($p < 0.001$) >P = CE ($p < 0.001$). The 50 Hz ELM-MF increased MNPCEs in mouse bone marrow, expressing a genotoxic capacity. Administration of antioxidant substances with radioprotective capacities known to act through the elimination of free radicals did not diminish the genotoxic effect induced by ELM-MF.

(E) Balamuralikrishnan B, Balachandar V, Kumar SS, Stalin N, Varsha P, Devi SM, Arun M, Manikantan P, Venkatesan C, Sasikala K, Dharwadkar SN. Evaluation of Chromosomal Alteration in Electrical Workers Occupationally Exposed to Low Frequency of Electro Magnetic Field (EMFs) in Coimbatore Population, India. Asian Pac J Cancer Prev. 13(6):2961-2966, 2012. (HU, LE, GT)

Extremely low frequency electromagnetic fields (EMFs) have been classified as possibly carcinogenic to humans by the International Agency for Research on Cancer. An increased number of chromosomal alterations in peripheral lymphocytes are correlated with elevated incidence of cancer. The aim of the present study was to assess occupationally induced chromosomal damage in EMF workers exposed to low levels of radiation. We used conventional metaphase chromosome aberration (CA) analysis and the micronucleus (MN) assay as biological indicators of nonionizing radiation exposure. In the present study totally 70 subjects were selected including 50 exposed and 20 controls. Informed written consent was obtained from all participants and the study was performed in accordance with the Declaration of Helsinki and the approval of the local ethical committee. A higher degree of CA and MN was observed in exposed subjects compared to controls, the frequency of CA being significantly enhanced with long years of exposure ($P<0.05$). Moreover increase in CA and MN with age was noted in both exposed subjects and controls, but was significantly greater in the former. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers occupationally exposed to EMFs in electric transformer and distribution stations. In conclusion, our findings suggest that EMFs possess genotoxic capability, as measured by CA and MN assays; CA analysis appeared more sensitive than other cytogenetic end-points. It can be concluded that chronic occupational exposure to EMFs may lead to an increased risk of genetic damage among electrical workers.

(E) Belyaev IY, Hillert L, Protopopova M, Tamm C, Malmgren LO, Persson BR, Selivanova G, Harms-Ringdahl M. 915 MHz microwaves and 50 Hz magnetic field affect chromatin conformation and 53BP1 foci in human lymphocytes from hypersensitive and healthy persons. Bioelectromagnetics 26:173-184, 2005. (GT, EH)

We used exposure to microwaves from a global system for mobile communication (GSM) mobile phone (915 MHz, specific absorption rate (SAR) 37 mW/kg) and power frequency magnetic field (50 Hz, 15 muT peak value) to investigate the response of lymphocytes from healthy subjects and from persons reporting hypersensitivity to electromagnetic field (EMF). The hypersensitive and healthy donors were matched by gender and age and the data were analyzed blind to treatment condition. The changes in chromatin conformation were measured with the method of anomalous viscosity time dependencies (AVTD). 53BP1 protein, which has been shown to colocalize in foci with DNA double strand breaks (DSBs), was analyzed by immunostaining in situ. Exposure at room temperature to either 915 MHz or 50 Hz resulted in significant condensation of chromatin, shown as AVTD changes, which was similar to the effect of heat shock at 41 degrees C. No significant differences in responses between normal and hypersensitive subjects were detected. Neither 915 MHz nor 50 Hz exposure induced 53BP1 foci. On the contrary, a distinct decrease in background level of 53BP1 signaling was observed upon these exposures as well as after heat shock treatments. This decrease correlated with the AVTD data and may indicate decrease in accessibility of 53BP1 to antibodies because of stress-induced chromatin condensation. Apoptosis was determined by morphological changes and by apoptotic fragmentation of DNA as analyzed by pulsed-field gel electrophoresis (PFGE). No apoptosis was induced by exposure to 50 Hz and 915 MHz microwaves. In conclusion, 50 Hz magnetic field and 915 MHz microwaves under specified conditions of exposure induced comparable responses in lymphocytes from healthy and hypersensitive donors that were similar but not identical to stress response induced by heat shock.

(E) Borhani N, Rajaei F, Salehi Z, Javadi A. Analysis of DNA fragmentation in mouse embryos exposed to an extremely low-frequency electromagnetic field. Electromagn Biol Med. 30(4):246-252, 2011. (GT, DE, LE)

Effects of extremely low-frequency electromagnetic fields (ELF-EMFs) on DNA damage in biological systems are still a matter of dispute. The aim of the present study was to investigate the possible effect of electromagnetic field exposure on DNA fragmentation in cells (blastomers) of mouse blastocysts. Eighty female NMRI mice were randomly divided into 2 groups of 40 animals each. The control group was left unexposed whereas the animals in the EMF-group were exposed to a 50-Hz EMF at 0.5 mT 4 h per day, 6 days a week for a duration of 2 weeks. After the 8(th) day of exposure, the female mice in both groups were superovulated (with injections of pregnant mare serum gonadotropin and human chorionic gonadotropin) and then mated overnight. At approximately 4 days after mating (102 h after the human chorionic gonadotropin treatment), blastocysts were obtained by flushing the uterus horns. The mean numbers of pregnant mice, blastocysts after flushing, blastomers within the blastocysts, and the DNA fragmentation index following staining in both groups were compared using statistical methods (SPSS, the Chi-square test, the Student's t-test and the Mann-Whitney U-test, $P < 0.05$). The results showed that the mean number of blastocysts after flushing was significantly decreased in the EMF-group compared to that of the control group ($P < 0.03$). The DNA fragmentation index was significantly increased in the EMF-group compared to control (10.53% vs. 7.14%; P <

0.001). However, there was no significant difference in the mean numbers of blastomers and numbers of pregnant mice between the EMF-exposed and control group. Our findings indicate that the EMF exposure in preimplantation stage could have detrimental effects on female mouse fertility and embryo development by decreasing the number of blastocysts and increasing the blastocysts DNA fragmentation.

(E) Bułdak RJ, Polaniak R, Bułdak L, Zwirska-Korczala K, Skonieczna M, Monsiol A, Kukla M, Duława-Bułdak A, Birkner E. Short-term exposure to 50 Hz ELF-EMF alters the cisplatin-induced oxidative response in AT478 murine squamous cell carcinoma cells. Bioelectromagnetics. 2012 Apr 25. doi: 10.1002/bem.21732. [Epub ahead of print] (GT, IA, OX)

The aim of this study was to assess the influence of cisplatin and an extremely low frequency electromagnetic field (ELF-EMF) on antioxidant enzyme activity and the lipid peroxidation ratio, as well as the level of DNA damage and reactive oxygen species (ROS) production in AT478 carcinoma cells. Cells were cultured for 24 and 72 h in culture medium with cisplatin. Additionally, the cells were irradiated with 50 Hz/1 mT ELF-EMF for 16 min using a solenoid as a source of the ELF-EMF. The amount of ROS, superoxide dismutase (SOD) isoenzyme activity, glutathione peroxidase (GSH-Px) activity, DNA damage, and malondialdehyde (MDA) levels were assessed. Cells that were exposed to cisplatin exhibited a significant increase in ROS and antioxidant enzyme activity. The addition of ELF-EMF exposure to cisplatin treatment resulted in decreased ROS levels and antioxidant enzyme activity. A significant reduction in MDA concentrations was observed in all of the study groups, with the greatest decrease associated with treatment by both cisplatin and ELF-EMF. Cisplatin induced the most severe DNA damage; however, when cells were also irradiated with ELF-EMF, less DNA damage occurred. Exposure to ELF-EMF alone resulted in an increase in DNA damage compared to control cells. ELF-EMF lessened the effects of oxidative stress and DNA damage that were induced by cisplatin; however, ELF-EMF alone was a mild oxidative stressor and DNA damage inducer. We speculate that ELF-EMF exerts differential effects depending on the exogenous conditions. This information may be of value for appraising the pathophysiologic consequences of exposure to ELF-EMF.

(E) Calabrò E, Condello S, Magazù S, Ientile, R. Static and 50 Hz electromagnetic fields effects on human neuronal-like cells vibration bands in the mid-infrared region. J Electromagnetic Analysis and Applications 3(2) 69-78, 2011. (GT)

Human neuronal-like cells were exposed to static and 50 Hz electromagnetic fields at the intensities of 2 mT and 1 mT, respectively. The effects of exposure were investigated in the mid-infrared region by means of Fourier self deconvolution spectroscopic analysis. After exposure of 3 hours to static and 50 Hz electromagnetic fields, the vibration bands of CH2 methilene group increased significantly after both exposures, suggesting a relative increase of lipid related to conformational changes in the cell membrane due to electromagnetic fields. In addition, PO2- stretching phosphate bands decreased after both exposures, suggesting that alteration in DNA/RNA can be occurred. In particular, exposure of 3 hours to 50 Hz electromagnetic fields produced significant increases in β-sheet contents in amide I, and around the 1740 cm^{-1} band assigned to non-hydrogen-bonded ester carbonyl stretching mode, that can be related to unfolding processes of proteins structure and cells death. Further exposure up to 18 hours to static magnetic field produced an increase in β-sheet contents as to α-helix components of amide I region, as well.

(E) Celikler S, Aydemir N, Vatan O, Kurtuldu S, Bilaloglu R. A biomonitoring study of genotoxic risk to workers of transformers and distribution line stations. Int J Environ Health Res. 19(6):421-430, 2009. (GT, HU)

A cytogenetic monitoring study was carried out on a group of workers from transformer and distribution line stations in the Bursa province of Turkey, to investigate the genotoxic risk of occupational exposure to extremely low frequency electric (ELF) and magnetic fields (EMF). Cytogenetic analysis, namely chromosomal aberrations (CAs) and micronucleus (MN) tests were performed on a strictly selected group of 55 workers and compared to 17 controls. CA and MN frequencies in electrical workers appeared significantly higher than in controls ($p < 0.001$, 0.05, respectively). The frequency of CA in exposed groups were significantly enhanced with the years of exposure ($p < 0.01$). The effect of smoking on the level of CA and MN was not significant in the control and exposure groups. The results of this study demonstrated that a significant induction of cytogenetic damage in peripheral lymphocytes of workers engaged to occupational exposure to ELMF in electric transformer and distribution stations.

(E) Chen GD, Lu DQ, Jiang H, Xu ZP.[Effects of 50 Hz magnetic fields on gene expression in MCF-7 cells]. Zhejiang Da Xue Xue Bao Yi Xue Ban. 37(1):15-22, 2008. [Article in Chinese] **(GT, GE)**

OBJECTIVE: To investigate whether 50 Hz magnetic fields (MF) can change the gene expression profile in MCF-7 cells and to screen MF responsive genes. **METHODS:** In vitro cultured MCF-7 cells were continuously exposed or sham-exposed to 0.4 mT of 50 Hz MF for 24 hours. Affymetrix Human Genome Genechips (U133A) were applied to analyze gene expression profiles in MF exposed and sham-exposed MCF-7 cells and the data were processed with Genechip data analysis software MAS 5.0 and DMT 3.0. Real-time RT-PCR assay was employed to examine the differentially expressed genes. **RESULT:** Thirty differentially expressed genes were screened with 100 % consistency change calls in the MF exposed MCF-7 cells. Six independent real-time RT-PCR analyses showed that SCNN1A, METTL3 and GPR137B were slightly but statistically significantly changed in MCF-7 cells after exposure to 50 Hz MF ($P<0.05$), while other analyzed genes exhibited slight up-and down-fluctuations in expressions and no increase or decrease in each gene expression reached statistical significance ($P>0.05$). **CONCLUSION:** The present study identified three 50 Hz MF responsive genes in MCF-7 cells and the biological consequences of expression changes in these MF responsive genes need to be further investigated.0.4 mT 50 Hz MF exposure for longer duration might induce DNA double-strand breaks in human lens epithelial cells in vitro.

(NE) Chen G, Lu D, Chiang H, Leszczynski D, Xu Z. Using model organism Saccharomyces cerevisiae to evaluate the effects of ELF-MF and RF-EMF exposure on global gene expression. Bioelectromagnetics. 33(7):550-560, 2012. (GE)

The potential health hazard of exposure to electromagnetic fields (EMF) continues to cause public concern. However, the possibility of biological and health effects of exposure to EMF remains controversial and their biophysical mechanisms are unknown. In the present study, we used Saccharomyces cerevisiae to identify genes responding to extremely low frequency magnetic fields (ELF-MF) and to radiofrequency EMF (RF-EMF) exposures. The yeast cells were exposed for 6 h to either 0.4 mT 50 Hz ELF-MF or 1800 MHz RF-EMF at a specific absorption rate of 4.7 W/kg. Gene expression was analyzed by microarray screening and confirmed using real-time reverse transcription-polymerase chain reaction (RT-PCR). We were unable to confirm microarray-detected changes in three of the ELF-MF responsive candidate genes using RT-PCR ($P > 0.05$). On the other hand, out of the 40 potential RF-EMF responsive genes, only the expressions of structural maintenance of chromosomes 3 (SMC3) and aquaporin 2 (AQY2 (m)) were confirmed, while three other genes, that is, halotolerance protein 9 (HAL9), yet another kinase 1 (YAK1) and one function-unknown gene (open reading frame: YJL171C), showed opposite changes in expression compared to the microarray data ($P < 0.05$). In conclusion, the results of this study suggest that the yeast cells did not alter gene expression in response to 50 Hz ELF-MF and that the response to RF-EMF is limited to only a very small number of genes. The possible biological consequences of the gene expression changes induced by RF-EMF await further investigation.

(E) Cho S, Lee Y, Lee S, Choi YJ, Chung HW. Enhanced cytotoxic and genotoxic effects of gadolinium following ELF-EMF irradiation in human lymphocytes. Drug Chem Toxicol. 2014 Jan 30. [Epub ahead of print] (GT, IA)

Gadolinium (Gd) and its chelated derivatives are widely utilized for various industrial and medical purposes, particularly as a contrast agent for magnetic resonance imaging (MRI). There are many studies of Gd nephrotoxicity and neurotoxicity, whereas research on cyto- and genotoxicity in normal human lymphocytes is scarce. It is important to investigate the effect of extremely low-frequency electromagnetic fields (ELF-EMF) on Gd toxicity, as patients are co-exposed to Gd and ELF-EMF generated by MRI scanners. We investigated the cytotoxicity and genotoixcity of Gd and the possible enhancing effect of ELF-EMF on Gd toxicity in cultured human lymphocytes by performing a micronuclei (MN) assay, trypan blue dye exclusion, single cell gel electrophoresis, and apoptosis analyses using flow cytometry. Isolated lymphocytes were exposed to 0.2-1.2 mM of Gd only or in combination with a 60-Hz ELF-EMF of 0.8-mT field strength. Exposing human lymphocytes to Gd resulted in a concentration- and time-dependent decrease in cell viability and an increase in MN frequency, single strand DNA breakage, apoptotic cell death, and ROS production. ELF-EMF (0.8 mT) exposure also increased cell death, MN frequency, olive tail moment, and apoptosis induced by Gd treatment alone. These results suggest that Gd induces DNA damage and apoptotic cell death in human lymphocytes and that ELF-EMF enhances the cytotoxicity and genotoxicity of Gd.

(E) Cho YH, Jeon HK, Chung HW. Effects of extremely low-frequency electromagnetic fields on delayed chromosomal instability induced by bleomycin in normal human fibroblast cells. J Toxicol Environ Health A. 70(15-16):1252-1258, 2007. (GT, IA)

This study was carried out to examine the interaction of extremely low-frequency electromagnetic fields (ELF-EMF) on delayed chromosomal instability by bleomycin (BLM) in human fibroblast cells. A micronucleus-centromere assay using DNA probes for chromosomes 1 and 4 was performed and a 60-Hz ELF-EMF of 0.8 mT field strength was applied either alone or with BLM throughout the culture period. The frequencies of micronuclei (MN) and aneuploidy were analyzed at 28, 88, and 240 h after treatment with BLM. The coexposure of cells to BLM and ELF-EMF led to a significant increase in the frequencies of MN and aneuploidy compared to the cells treated with BLM alone. No difference was observed between field-exposed and sham-exposed control cells. The frequency of MN induced by BLM was increased at 28 h, and further analysis showed a persistent increase up to 240 h, but the new levels were not significantly different from the level at 28 h. BLM increased the frequencies of aneuploidy at 28, 88, and 240 h, and significantly higher frequency of aneuploidy was observed in the cells analyzed at 240 h compared to the cells examined at 28 h. No interaction of ELF-EMF on delayed chromosomal instability by BLM was observed. Our results suggest that ELF-EMF enhances the cytotoxicity of BLM. BLM might induce delayed chromosomal instability, but no effect of ELF-EMF was observed on the BLM-induced delayed chromosomal instability in fibroblast cells.

(E) Collard JF, Lazar C, Nowé A, Hinsenkamp M. Statistical validation of the acceleration of the differentiation at the expense of the proliferation in human epidermal cells exposed to extremely low frequency electric fields. Prog Biophys Mol Biol. 111(1):37-45, 2013. (GE)

An acceleration of differentiation at the expense of proliferation is observed in our previous publications and in the literature after exposure of various biological models to low frequency and low-amplitude electric and electromagnetic fields. This observation is related with a significant modification of genes expression. We observed and compared over time this modification. This study use microarray data obtained on epidermis cultures harvested from human abdominoplasty exposed to ELF electric fields. This protocol is repeated with samples collected on three different healthy patients. The sampling over time allows comparison of the effect of the stimulus at a given time with the evolution of control group. After 4 days, we observed a significant difference of the genes expression between control (D4C) and stimulated (D4S) ($p < 0.05$). On the control between day 4 and 7, we observed another group of genes with significant difference ($p < 0.05$) in their expression. We identify the common genes between these two groups and we select from them those expressing no difference between stimulate at 4 days (D4S) and control after 7 days (D7C). The same analysis was performed with D4S-D4C-D12C and D7S-D7C-D12C. The lists of genes which follow this pattern show acceleration in their expressions under stimulation appearing on control at a later time. In this list, genes such as DKK1, SPRR3, NDRG4, and CHEK1 are involved in cell proliferation or differentiation. Numerous other genes are also playing a function in mitosis, cell cycle or in the DNA replication transcription and translation.

(E) Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C, Azzena GB, Grassi C.
Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. Exp Neurol. 226(1):173-182, 2010. (LE, GE, DE)

Throughout life, new neurons are continuously generated in the hippocampus, which is therefore a major site of structural plasticity in the adult brain. We recently demonstrated that extremely low-frequency electromagnetic fields (ELFEFs) promote the neuronal differentiation of neural stem cells in vitro by up-regulating Ca(v)1-channel activity. The aim of the present study was to determine whether 50-Hz/1 mT ELFEF stimulation also affects adult hippocampal neurogenesis in vivo, and if so, to identify the molecular mechanisms underlying this action and its functional impact on synaptic plasticity. ELFEF exposure (1 to 7 h/day for 7 days) significantly enhanced neurogenesis in the dentate gyrus (DG) of adult mice, as documented by increased numbers of cells double-labeled for 5-bromo-deoxyuridine (BrdU) and double cortin. Quantitative RT-PCR analysis of hippocampal extracts revealed significant ELFEF exposure-induced increases in the transcription of pro-neuronal genes (Mash1, NeuroD2, Hes1) and genes encoding Ca(v)1.2 channel α(1C) subunits. Increased expression of NeuroD1, NeuroD2 and Ca(v)1 channels was also documented by Western blot analysis. Immunofluorescence experiments showed that, 30 days after ELFEF stimulation, roughly half of the newly generated immature neurons had survived and become mature dentate granule cells (as shown by their immunoreactivity for both BrdU and NeuN) and were integrated into the granule cell layer of the DG. Electrophysiological experiments demonstrated that the new mature neurons influenced hippocampal synaptic plasticity, as reflected by increased long-term potentiation. Our findings show that ELFEF exposure can be an effective tool for increasing in vivo neurogenesis, and they could lead to the development of novel therapeutic approaches in regenerative medicine.

(E) Di Campli E, Di Bartolomeo S, Grande R, Di Giulio M, Cellini L. Effects of extremely low-frequency electromagnetic fields on Helicobacter pylori biofilm. Curr Microbiol. 60(6):412-418, 2010. (GE)

The aim of this work was to investigate the effects of exposure to extremely low-frequency electromagnetic fields (ELF-EMF) both on biofilm formation and on mature biofilm of Helicobacter pylori. Bacterial cultures and 2-day-old biofilm of H. pylori ATCC 43629 were exposed to ELF-EMF (50 Hz frequency-1 mT intensity) for 2 days to assess their effect on the cell adhesion and on the mature biofilm detachment, respectively. All the exposed cultures and the respective sham exposed controls were studied for: the cell viability status, the cell morphological analysis, the biofilm mass measurement, the genotypic profile, and the luxS and amiA gene expression. The ELF-EMF acted on the bacterial population during the biofilm formation displaying significant differences in cell viability, as well as, in morphotypes measured by the prevalence of spiral forms (58.41%) in respect to the controls (33.14%), whereas, on mature biofilm, no significant differences were found when compared to the controls. The measurement of biofilm cell mass was significantly reduced in exposed cultures in both examined experimental conditions. No changes in DNA patterns were recorded, whereas a modulation in amiA gene expression was detected. An exposure to ELF-EMF of H. pylori biofilm induces phenotypic changes on adhering bacteria and decreases the cell adhesion unbalancing the bacterial population therefore reducing the H. pylori capability to protect itself.

(E) Dominici L, Villarini M, Fatigoni C, Monarca S, Moretti M. Genotoxic hazard evaluation in welders occupationally exposed to extremely low-frequency magnetic fields (ELF-MF). Int J Hyg Environ Health. 215(1):68-75, 2011. (GT, HU)

Electric arc welding is known to involve considerable exposure to extremely low-frequency magnetic fields (ELF-MF). A cytogenetic monitoring study was carried out in a group of welders to investigate the genotoxic risk of occupational exposure to ELF-MF. This study assessed individual occupational exposure to ELF-MF using a personal magnetic-field dosimeter, and the cytogenetic effects were examined by comparing micronuclei (MN) and sister chromatid exchange (SCE) frequencies in the lymphocytes of the exposed workers with those of non-exposed control subjects (blood donors) matched for age and smoking habit. Cytogenetic analyses were carried out on 21 workers enrolled from two different welding companies in Central Italy and compared to 21 controls. Some differences between the groups were observed on analysis of SCE and MN, whereas replication indices in the exposed were found not to differ from the controls. In particular, the exposed group showed a significantly higher frequency of MN (group mean±SEM: 6.10±0.39) compared to the control group (4.45±0.30). Moreover, the increase in MN is associated with a proportional increase in ELF-MF exposure levels with a dose-response relationship. A significant decrease in SCE frequency was observed in exposed subjects (3.73±0.21) compared to controls (4.89±0.12). The hypothesis of a correlation between genotoxic assays and ELF-MF exposure value was partially supported, especially as regards MN assay. Since these results are derived from a small-scale pilot study, a larger scale study should be undertaken.

(E) Du XG, Xu SS, Chen Q, Lu DQ, Xu ZP, Zeng QL. [Effects of 50 Hz magnetic fields on DNA double-strand breaks in human lens epithelial cells]. Zhejiang Da Xue Xue Bao Yi Xue Ban. 37(1):9-14, 2008. [Article in Chinese] (GT)

OBJECTIVE: To investigate the effects of 50 Hz magnetic fields (MF) on DNA double-strand breaks in human lens epithelial cells (hLECs). **METHODS:** The cultured human lens epithelial cells were exposed to 0.4 mT 50 Hz MF for 2 h, 6 h, 12 h, 24 h and 48 h. Cells exposed to 4-nitroquinoline-1-oxide, a DNA damage agent, at a final concentration of 0.1 micromol/L for 1 h were used as positive controls.After exposure, cells were fixed with 4 % paraformaldehyde and for H2AX (gamma H2AX) immunofluorescence measurement. gamma H2AX foci were detected at least 200 cells for each sample. Cells were classified as positive when more than three foci per cell were observed. Mean values of foci per cell and percentage of foci positive cells were adopted as indexes of DNA double-strand breaks. **RESULT:** The mean value of foci per cell and the percentage of gamma H2AX foci positive cells in 50 Hz MF exposure group for 24 h were (2.93 +/-0.43) and (27.88 +/-2.59)%, respectively, which were significantly higher than those of sham-exposure group [(1.77 +/-0.37) and (19.38+/-2.70)%, P <0.05], and the mean value of foci per cell and the percentage of gamma H2AX foci positive cells in 50 Hz MF exposure group for 48 h were (3.14 +/-0.35) and (31.00 +/-3.44)%, which were significantly higher than those of sham-exposure group (P <0.01). However there was no significant difference between 50 Hz MF exposure groups for 2 h, 6 h, 12 h and sham-exposure group for above two indexes (P >0.05). **CONCLUSION:** 0.4 mT 50 Hz MF exposure for longer duration might induce DNA double-strand breaks in human lens epithelial cells in vitro.

(E) El-Bialy NS, Rageh MM. Extremely low-frequency magnetic field enhances the therapeutic efficacy of low-dose cisplatin in the treatment of Ehrlich carcinoma. Biomed Res Int. 2013;2013:189352. doi: 10.1155/2013/189352. Epub 2013 Jan 14. (GT, IA)

The present study examines the therapeutic efficacy of the administration of low-dose cisplatin (cis) followed by exposure to extremely low-frequency magnetic field (ELF-MF), with an average intensity of 10 mT, on Ehrlich carcinoma in vivo. The cytotoxic and genotoxic actions of this combination were studied using comet assay, mitotic index (MI), and the induction of micronucleus (MN). Moreover, the inhibition of tumor growth was also measured. Treatment with cisplatin and ELF-MF (group A) increased the number of damaged cells by 54% compared with 41% for mice treated with cisplatin alone (group B), 20% for mice treated by exposure to ELF-MF (group C), and 9% for the control group (group D). Also the mitotic index decreased significantly for all treated groups ($P < 0.001$). The decrement percent for the treated groups (A, B, and C) were 70%, 65%, and 22%, respectively, compared with the control group (D). Additionally, the rate of tumor growth at day 12 was suppressed significantly ($P < 0.001$) for groups A, B, and C with respect to group (D). These results suggest that ELF-MF enhanced the cytotoxic activity of cisplatin and potentiate the benefit of using a combination of low-dose cisplatin and ELF-MF in the treatment of Ehrlich carcinoma.

(E) Erdal N, Gürgül S, Celik A. Cytogenetic effects of extremely low frequency magnetic field on Wistar rat bone marrow. Mutat Res. 630(1-2):69-77, 2007. (GT, LE)

In this study, the genotoxic and cytotoxic potential of extremely low frequency magnetic fields (ELF-MF) was investigated in Wistar rat tibial bone marrow cells, using the chromosomal aberration (CA) and micronucleus (MN) test systems. In addition to these test systems, we also investigated the mitotic index (MI), and the ratio of polychromatic erythrocytes (PCEs) to normochromatic erythrocytes (NCEs). Wistar rats were exposed to acute (1 day for 4h) and long-term (4h/day for 45 days) to a horizontal 50Hz, 1mT uniform magnetic field generated by a Helmholtz coil system. Mitomycin C (MMC, 2mg/kg BW) was used as positive control. Results obtained by chromosome analysis do not show any statistically significant differences between the negative control and both acute and long-term ELF-MF exposed samples. When comparing the group mean CA of long-term exposure with the negative control and acute exposure, the group mean of the long-term exposed group was higher, but this was not statistically significant. However, the mean micronucleus frequency of the longer-term exposed group was considerably higher than the negative control and acutely exposed groups. This difference was statistically significant ($p<0.01$). The results of the MI in bone marrow showed that the averages of both A-MF and L-MF groups significantly decreased when compared to those in the negative control ($p<0.001$ and $p<0.01$, respectively). No significant differences were found between the group mean MI of A-MF exposure with L-MF. We found that the average of PCEs/NCEs ratios of A-MF exposed group was significantly lower than the negative control and L-MF exposed groups ($p<0.001$ and $p<0.01$, respectively). In addition, the group mean of the PCEs/NCEs ratios of L-MF was significantly lower than negative control ($p<0.01$). We also found that the MMC treated group showed higher the number of CA and the frequency of MN formation when compared to those in all other each groups (p-values of all each groups <0.01) and also MMC treated group showed lower MI and the PCEs/NCEs ratios when compared to those in all other each groups (p-values of all groups <0.01). These observations indicate the in vivo suspectibility of mammals to the genotoxicity potential of ELF-MF.

(E) Fedrowitz M, Löscher W. Gene expression in the mammary gland tissue of female Fischer 344 and Lewis rats after magnetic field exposure (50 Hz, 100 µT) for 2 weeks. Int J

Radiat Biol. 88(5):425-429, 2012. (GE, LE) See also: Fedrowitz M, Hass R, Löscher W. Effects of 50 Hz magnetic field exposure on the stress marker α-amylase in the rat mammary gland. Int J Radiat Biol. 88(7):556-564, 2012.

PURPOSE: The issue of whether exposure to environmental power-frequency magnetic fields (MF) has impact on breast cancer development still remains equivocal. Previously, we observedrat strain differences in the MF response of breast tissue, so that the genetic background plays a role in MF effects. The present experiment aimed to elucidate candidate genes involved in MF effects by comparison of MF-susceptible Fischer 344 (F344) rats and MF-insensitive Lewis rats. **MATERIALS AND METHODS:** Female F344 and Lewis rats were exposed to MF (50 Hz, 100 µT) for two weeks, and a whole genome microarray analysis in the mammary gland tissue was performed. **RESULTS:** A remarkably decreased α-amylase gene expression, decreases in carbonic anhydrase 6 and lactoperoxidase, both relevant for pH regulation, and an increased gene expression of cystatin E/M, a tumor suppressor, were observed in MF-exposed F344, but not in Lewis rats. **CONCLUSION:** The MF-exposed F344 breast tissue showed alterations in gene expression, which were absent in Lewis and may therefore be involved in the MF-susceptibility of F344. Notably α-amylase might serve as a promising target to study MF effects, because first experiments indicate that MF exposure alters the functionality of this enzyme in breast tissue.

(E) Focke F, Schuermann D, Kuster N, Schär P. DNA fragmentation in human fibroblasts under extremely low frequency electromagnetic field exposure. Mutat Res. 683(1-2):74-83, 2010. (GT)

Extremely low frequency electromagnetic fields (ELF-EMFs) were reported to affect DNA integrity in human cells with evidence based on the Comet assay. These findings were heavily debated for two main reasons; the lack of reproducibility, and the absence of a plausible scientific rationale for how EMFs could damage DNA. Starting out from a replication of the relevant experiments, we performed this study to clarify the existence and explore origin and nature of ELF-EMF induced DNA effects. Our data confirm that intermittent (but not continuous) exposure of human primary fibroblasts to a 50 Hz EMF at a flux density of 1 mT induces a slight but significant increase of DNA fragmentation in the Comet assay, and we provide first evidence for this to be caused by the magnetic rather than the electric field. Moreover, we show that EMF-induced responses in the Comet assay are dependent on cell proliferation, suggesting that processes of DNA replication rather than the DNA itself may be affected. Consistently, the Comet effects correlated with a reduction of actively replicating cells and a concomitant increase of apoptotic cells in exposed cultures, whereas a combined Fpg-Comet test failed to produce evidence for a notable contribution of oxidative DNA base damage. Hence, ELF-EMF induced effects in the Comet assay are reproducible under specific conditions and can be explained by minor disturbances in S-phase processes and occasional triggering of apoptosis rather than by the generation of DNA damage.

(E) Frisch P, Li GC, McLeod K, Laramee CB. Induction of heat shock gene expression in RAT1 primary fibroblast cells by ELF electric fields. Bioelectromagnetics. 34(5):405-413, 2013. (GE)

Recent studies have demonstrated that the Ku70 gene fragment can be placed in the anti-sense orientation under the control of a heat-inducible heat shock protein 70 (HSP70) promoter and activated through heat shock exposure. This results in attenuation of the Ku70 protein expression, inhibiting cellular repair processes, and sensitizing the transfected cells to exposures such as the ionizing radiation exposures used clinically. However, achieving the tissue temperatures necessary to thermally induce the HSP70 response presents significant limitations to the clinical application of this strategy. Previous findings suggest an alternative approach to inducing a heat shock response, specifically through the use of extremely low frequency (ELF) electrical field stimulation. To further pursue this approach, we investigated HSP70 responses in transfected rat primary fibroblast (RAT1) cells exposed to 10 Hz electric fields at intensities of 20-500 V/m. We confirmed that low frequency electric fields can induce HSP70 heat shock expression, with peak responses obtained at 8 h following a 2 h field exposure. However, the approximate threefold increase in expression is substantially lower than that obtained using thermal stimulation, raising questions of the clinical utility of the response.

(E) Giorgi G, Marcantonio P, Bersani F, Gavoçi E, Del Re B. Effect of extremely low frequency magnetic field exposure on DNA transposition in relation to frequency, wave shape and exposure time. Int J Radiat Biol. 87(6):601-608, 2011. (GT, WS)

PURPOSE: To examine the effect of extremely low frequency magnetic field (ELF-MF) exposure on transposon (Tn) mobility in relation to the exposure time, the frequency and the wave shape of the field applied. **MATERIALS AND METHODS:** Two Escherichia coli model systems were used: (1) Cells unable to express β-galactosidase (LacZ(-)), containing a mini-transposon Tn10 element able to give ability to express β-galactosidase (LacZ(+)) upon its transposition; therefore in these cells transposition activity can be evaluated by analysing LacZ(+) clones; (2) cells carrying Fertility plasmid (F(+)), and a Tn5 element located on the chromosome; therefore in these cells transposition activity can be estimated by a bacterial conjugation assay. Cells were exposed to sinusoidal (SiMF) or pulsed-square wave (PMF) magnetic fields of various frequencies (20, 50, 75 Hz) and for different exposure times (15 and 90 min). **RESULTS:** Both mini-Tn10 and Tn5 transposition decreased under SiMF and increased under PMF, as compared to sham exposure control. No significant difference was found between frequencies and between exposure times. **CONCLUSIONS:** ELF-MF exposure affects transposition activity and the effects critically depend on the wave shape of the field, but not on the frequency and the exposure time, at least in the range observed.

(E) Heredia-Rojas JA, Rodríguez de la Fuente AO, Alcocer González JM, Rodríguez-Flores LE, Rodríguez-Padilla C, Santoyo-Stephano MA, Castañeda-Garza E, Taméz-Guerra RS. Effect of 60 Hz magnetic fields on the activation of hsp70 promoter in cultured INER-37 and RMA E7 cells. In Vitro Cell Dev Biol Anim. 46(9):758-63, 2010. (GE)

It has been reported that 50-60 Hz magnetic fields (MF) with flux densities ranging from microtesla to millitesla are able to induce heat shock factor or heat shock proteins in various cells. In this study, we investigated the effect of 60 Hz sinusoidal MF at 8 and 80 µT on the expression of the luciferase gene contained in a plasmid labeled as electromagnetic field-plasmid (pEMF). This gene construct contains the specific sequences previously described for the induction of hsp70 expression by MF, as well as the reporter for the luciferase gene. The pEMF vector was transfected into INER-37 and RMA E7 cell lines that were later exposed to either MF or thermal shock (TS). Cells that received the MF or TS treatments and their controls were processed according to the luciferase assay system for evaluate luciferase activity. An increased luciferase gene expression was observed in INER-37 cells exposed to MF and TS compared with controls ($p < 0.05$), but MF exposure had no effect on the RMA E7 cell line.

(NE) Huwiler SG, Beyer C, Fröhlich J, Hennecke H, Egli T, Schürmann D, Rehrauer H, Fischer HM. Genome-wide transcription analysis of Escherichia coli in response to extremely low-frequency magnetic fields. Bioelectromagnetics. 2012 Feb 13. doi: 10.1002/bem.21709. [Epub ahead of print] (GE)

The widespread use of electricity raises the question of whether or not 50 Hz (power line frequency in Europe) magnetic fields (MFs) affect organisms. We investigated the transcription of Escherichia coli K-12 MG1655 in response to extremely low-frequency (ELF) MFs. Fields generated by three signal types (sinusoidal continuous, sinusoidal intermittent, and power line intermittent; all at 50 Hz, 1 mT) were applied and gene expression was monitored at the transcript level using an Affymetrix whole-genome microarray. Bacterial cells were grown continuously in a chemostat (dilution rate $D = 0.4\ h(-1)$) fed with glucose-limited minimal medium and exposed to 50 Hz MFs with a homogenous flux density of 1 mT. For all three types of MFs investigated, neither bacterial growth (determined using optical density) nor culturable counts were affected. Likewise, no statistically significant change (fold-change > 2, $P \leq 0.01$) in the expression of 4,358 genes and 714 intergenic regions represented on the gene chip was detected after MF exposure for 2.5 h (1.4 generations) or 15 h (8.7 generations). Moreover, short-term exposure (8 min) to the sinusoidal continuous and power line intermittent signal neither affected bacterial growth nor showed evidence for reliable changes in transcription. In conclusion, our experiments did not indicate that the different tested MFs (50 Hz, 1 mT) affected the transcription of E. coli.

(NE) Jin YB, Kang GY, Lee JS, Choi JI, Lee JW, Hong SC, Myung SH, Lee YS. Effects on micronuclei formation of 60-Hz electromagnetic field exposure with ionizing radiation, hydrogen peroxide, or c-Myc overexpression. Int J Radiat Biol. 88(4):374-380, 2012. (GT, IA)

PURPOSE: Epidemiological studies have demonstrated a possible correlation between exposure to extremely low-frequency magnetic fields (ELF-MF) and cancer. However, this correlation has yet to be definitively confirmed by epidemiological studies. The principal objective of this study was to assess the effects of 60 Hz magnetic fields in a normal cell line system, and particularly in combination with various external factors, via micronucleus (MN) assays. **MATERIALS AND METHODS:** Mouse embryonic fibroblast NIH3T3 cells and human lung fibroblast WI-38 cells were exposed for 4 h to a 60 Hz, 1 mT uniform magnetic field with or without ionizing radiation (IR, 2 Gy), $H(2)O(2)$ (100 µM) and cellular myelocytomatosis oncogene (c-Myc) activation. **RESULTS:** The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic effects were observed when ELF-MF was combined with IR, $H(2)O(2)$, and c-Myc activation. **CONCLUSIONS:** Our results demonstrate that ELF-MF did not enhance MN frequency by IR, H(2)O(2) and c-Myc activation.

(NE) Jin YB, Choi SH, Lee JS, Kim JK, Lee JW, Hong SC, Myung SH, Lee YS. Absence of DNA damage after 60-Hz electromagnetic field exposure combined with ionizing radiation, hydrogen peroxide, or c-Myc overexpression. Radiat Environ Biophys. 2013 Dec 5. [Epub ahead of print] (GT, IA)

The principal objective of this study was to assess the DNA damage in a normal cell line system after exposure to 60 Hz of extremely low frequency magnetic field (ELF-MF) and particularly in combination with various external factors, via comet assays. NIH3T3 mouse fibroblast cells, WI-38 human lung fibroblast cells, L132 human lung epithelial cells, and MCF10A human mammary gland epithelial cells were exposed for 4 or 16 h to a 60-Hz, 1 mT uniform magnetic field in the presence or absence of ionizing radiation (IR, 1 Gy), H_2O_2 (50 μM), or c-Myc oncogenic activation. The results obtained showed no significant differences between the cells exposed to ELF-MF alone and the unexposed cells. Moreover, no synergistic or additive effects were observed after 4 or 16 h of pre-exposure to 1 mT ELF-MF or simultaneous exposure to ELF-MF combined with IR, H_2O_2, or c-Myc activation.

(E) Jouni FJ, Abdolmaleki P, Ghanati F. Oxidative stress in broad bean (Vicia faba L.) induced by static magnetic field under natural radioactivity. Mutat Res. 741(1-2):116-121, 2012. (LE, GT, OX, IA)

The investigation was performed to evaluate the influence of the static magnetic field on oxidative stress in Vicia faba cultivated in soil from high background natural radioactivity in Iran. Soil samples were collected from Ramsar, Iran where the annual radiation absorbed dose from background radiation is substantially higher than 20 mSv/year. The soil samples were then divided into 2 separate groups including high and low natural radioactivity. The plants were continuously exposed to static magnetic field of 15 mT for 8 days, each 8h/day. The results showed that in the plants cultivated in soils with high background natural radioactivity and low background natural radioactivity the activity of antioxidant enzymes as well as flavonoid content were lower than those of the control. Treatment of plants with static magnetic field showed similar results in terms of lowering of antioxidant defense system and increase of peroxidation of membrane lipids. Accumulation of ROS also resulted in chromosomal aberration and DNA damage. This phenomenon was more pronounced when a combination of natural radiation and treatment with static magnetic field was applied. The results suggest that exposure to static magnetic field causes accumulation of reactive oxygen species in V. faba and natural radioactivity of soil exaggerates oxidative stress.

(E) Kim J, Ha CS, Lee HJ, Song K. Repetitive exposure to a 60-Hz time-varying magnetic field induces DNA double-strand breaks and apoptosis in human cells. Biochem Biophys Res Commun. 400(4):739-744, 2010. (GT)

We investigated the effects of extremely low frequency time-varying magnetic fields (MFs) on human normal and cancer cells. Whereas a single exposure to a 60-Hz time-varying MF of 6 mT for 30min showed no effect, repetitive exposure decreased cell viability. This decrease was accompanied by phosphorylation of γ-H2AX, a common DNA double-strand break (DSB) marker, and checkpoint kinase 2 (Chk2), which is critical to the DNA damage checkpoint pathway. In addition, repetitive exposure to a time-varying MF of 6 mT for 30 min every 24 h for 3 days led to p38 activation and induction of apoptosis in cancer and normal cells. Therefore, these results demonstrate that repetitive exposure to MF with extremely low frequency can induce DNA DSBs and apoptosis through p38 activation. These results also suggest the need for further evaluation of the effects of repetitive exposure to environmental time-varying MFs on human health.

(E) Kim J, Yoon Y, Yun S, Park GS, Lee HJ, Song K. Time-varying magnetic fields of 60 Hz at 7 mT induce DNA double-strand breaks and activate DNA damage checkpoints without apoptosis. Bioelectromagnetics. 33(5):383-393, 2012. (GT, WS)

The potential genotoxic effect of a time-varying magnetic field (MF) on human cells was investigated. Upon continuous exposure of human primary fibroblast and cervical cancer cells to a 60 Hz MF at 7 mT for 10-60 min, no significant change in cell viability was observed. However, deoxyribonucleic acid (DNA) double-strand breaks (DSBs) were detected, and the DNA damage checkpoint pathway was activated in these cells without programmed cell death (called apoptosis). The exposure of human cells to a 60 Hz MF did not induce intracellular reactive oxygen species (ROS) production, suggesting that the observed DNA DSBs are not directly caused by ROS. We also compared the position and time dependency of DNA DSBs with numerical simulation of MFs. The Lorentz force and eddy currents in these experiments were numerically calculated to investigate the influence of each factor on DNA DSBs. The DNA DSBs mainly occurred at the central region, where the MF was strongest, after a 30-min exposure. After 90 min, however, the amount of DNA DSBs increased rapidly in the outer regions, where the eddy current and Lorentz force were strong.

(NE) Kirschenlohr H, Ellis P, Hesketh R, Metcalfe J. Gene Expression Profiles in White Blood Cells of Volunteers Exposed to a 50 Hz Electromagnetic Field. Radiat Res. 178(3): 138-149, 2012. (GE, HU)

Consistent and independently replicated laboratory evidence to support a causative relationship between environmental exposure to extremely low-frequency electromagnetic fields (EMFs) at power line frequencies and the associated increase in risk of childhood leukemia has not been obtained. In particular, although gene expression responses have been reported in a wide variety of cells, none has emerged as robust, widely replicated effects. DNA microarrays facilitate comprehensive searches for changes in gene expression without a requirement to select candidate responsive genes. To determine if gene expression changes occur in white blood cells of volunteers exposed to an ELF-EMF, each of 17 pairs of male volunteers age 20-30 was subjected either to a 50 Hz EMF exposure of 62.0 ± 7.1 μT for 2 h or to a sham exposure (0.21 ± 0.05 μT) at the same time (11:00 a.m. to 13:00 p.m.). The alternative regime for each volunteer was repeated on the following day and the two-day sequence was repeated 6 days later, with the exception that a null exposure (0.085 ± 0.01 μT) replaced the sham exposure. Five blood samples (10 ml) were collected at 2 h intervals from 9:00 to 17:00 with five additional samples during the exposure and sham or null exposure periods on each study day. RNA samples were pooled for the same time on each study day for the group of 17 volunteers that were subjected to the

ELF-EMF exposure/sham or null exposure sequence and were analyzed on Illumina microarrays. Time courses for 16 mammalian genes previously reported to be responsive to ELF-EMF exposure, including immediate early genes, stress response, cell proliferation and apoptotic genes were examined in detail. No genes or gene sets showed consistent response profiles to repeated ELF-EMF exposures. A stress response was detected as a transient increase in plasma cortisol at the onset of either exposure or sham exposure on the first study day. The cortisol response diminished progressively on subsequent exposures or sham exposures, and was attributable to mild stress associated with the experimental protocol.

(E) Koyama S, Sakurai T, Nakahara T, Miyakoshi J. Extremely low frequency (ELF) magnetic fields enhance chemically induced formation of apurinic/apyrimidinic (AP) sites in A172 cells. Int J Radiat Biol. 84(1):53-59, 2008. (GT, IA)

PURPOSE: To detect the effects of extremely low frequency (ELF) magnetic fields, the number of apurinic/apyrimidinic (AP) sites in human glioma A172 cells was measured following exposure to ELF magnetic fields. **MATERIALS AND METHODS:** The cells were exposed to an ELF magnetic field alone, to genotoxic agents (methyl methane sulfonate (MMS) and hydrogen peroxide (H_2O_2)) alone, or to an ELF magnetic field with the genotoxic agents. After exposure, DNA was extracted, and the number of AP sites was measured. **RESULTS:** There was no difference in the number of AP sites between cells exposed to an ELF magnetic field and sham controls. With MMS or H_2O_2 alone, the number of AP sites increased with longer treatment times. Exposure to an ELF magnetic field in combination with the genotoxic agents increased AP-site levels compared with the genotoxic agents alone. **CONCLUSIONS:** Our results suggest that the number of AP sites induced by MMS or H_2O_2 is enhanced by exposure to ELF magnetic fields at 5 millitesla (mT). This may occur because such exposure can enhance the activity or lengthen the lifetime of radical pairs.

(E) Lee JW, Kim MS, Kim YJ, Choi YJ, Lee Y, Chung HW. Genotoxic effects of 3 T magnetic resonance imaging in cultured human lymphocytes. Bioelectromagnetics. 32(7):535-542, 2011. (GT)

The clinical and preclinical use of high-field intensity (HF, 3 T and above) magnetic resonance imaging (MRI) scanners have significantly increased in the past few years. However, potential health risks are implied in the MRI and especially HF MRI environment due to high-static magnetic fields, fast gradient magnetic fields, and strong radiofrequency electromagnetic fields. In this study, the genotoxic potential of 3 T clinical MRI scans in cultured human lymphocytes in vitro was investigated by analyzing chromosome aberrations (CA), micronuclei (MN), and single-cell gel electrophoresis. Human lymphocytes were exposed to electromagnetic fields generated during MRI scanning (clinical routine brain examination protocols: three-channel head coil) for 22, 45, 67, and 89 min. We observed a significant increase in the frequency of single-strand DNA breaks following exposure to a 3 T MRI. In addition, the frequency of both CAs and MN in exposed cells increased in a time-dependent manner. The frequencies of MN in lymphocytes exposed to complex electromagnetic fields for 0, 22, 45, 67, and 89 min were 9.67, 11.67, 14.67, 18.00, and 20.33 per 1000 cells, respectively. Similarly, the frequencies of CAs in lymphocytes exposed for 0, 45, 67, and 89 min were 1.33, 2.33, 3.67, and 4.67 per 200 cells, respectively. These results suggest that exposure to 3 T MRI induces genotoxic effects in human lymphocytes.

(E) Leone L, Fusco S, Mastrodonato A, Piacentini R, Barbati SA, Zaffina S, Pani G, Podda MV, Grassi C. Epigenetic Modulation of Adult Hippocampal Neurogenesis by Extremely Low-Frequency Electromagnetic Fields. Mol Neurobiol. 2014 Feb 16. [Epub ahead of print] (GE)

Throughout life, adult neurogenesis generates new neurons in the dentate gyrus of hippocampus that have a critical role in memory formation. Strategies able to stimulate this endogenous process have raised considerable interest because of their potential use to treat neurological disorders entailing cognitive impairment. We previously reported that mice exposed to extremely low-frequency electromagnetic fields (ELFEFs) showed increased hippocampal neurogenesis. Here, we demonstrate that the ELFEF-dependent enhancement of hippocampal neurogenesis improves spatial learning and memory. To gain insights on the molecular mechanisms underlying ELFEFs' effects, we extended our studies to an in vitro model of neural stem cells (NSCs) isolated from the hippocampi of newborn mice. We found that ELFEFs enhanced proliferation and neuronal differentiation of hippocampal NSCs by regulation of epigenetic mechanisms leading to pro-neuronal gene expression. Upon ELFEF stimulation of NSCs, we observed a significant enhancement of expression of the pro-proliferative gene hairy enhancer of split 1 and the neuronal determination genes NeuroD1 and Neurogenin1. These events were preceded by increased acetylation of H3K9 and binding of the phosphorylated transcription factor cAMP response element-binding protein (CREB) on the regulatory sequence of these genes. Such ELFEF-dependent epigenetic modifications were prevented by the Ca_v1-channel blocker nifedipine, and were associated with increased occupancy of CREB-binding protein (CBP) to the same loci within the analyzed promoters. Our results unravel the molecular mechanisms underlying the ELFEFs' ability to improve endogenous neurogenesis, pointing to histone acetylation-related chromatin remodeling as a critical determinant. These findings could pave the way to the development of novel therapeutic approaches in regenerative medicine.

(E) Li SS, Zhang ZY, Yang CJ, Lian HY, Cai P. Gene expression and reproductive abilities of male Drosophila melanogaster subjected to ELF-EMF exposure. Mutat Res. 758(1-2):95-103, 2013. (GE, LE, RP)

Extremely low frequency electromagnetic field (ELF-EMF) exposure is attracting increased attention as a possible disease-inducing factor. The in vivo effects of short-term and long-term ELF-EMF exposure on male Drosophila melanogaster were studied using transcriptomic analysis for preliminary screening and QRT-PCR for further verification. Transcriptomic analysis indicated that 439 genes were up-regulated and 874 genes were down-regulated following short-term exposures and that 514 genes were up-regulated and 1206 genes were down-regulated following long-term exposures (expression >2- or <0.5-fold, respectively). In addition, there are 238 up-regulated genes and 598 down-regulated genes in the intersection of short-term and long-term exposure (expression >2- or <0.5-fold). The DEGs (differentially expressed genes) in D. melanogaster following short-term exposures were involved in metabolic processes, cytoskeletal organization, mitotic spindle organization, cell death, protein modification and proteolysis. Long-term exposure let to changes in expression of genes involved in metabolic processes, response to stress, mitotic spindle organization, aging, cell death and cellular respiration. In the intersection of short-term and long-term exposure, a series of DEGs were related to apoptosis, aging, immunological stress and reproduction. To check the ELF-EMF effects on reproduction, some experiments on male reproduction ability were performed. Their results indicated that short-term ELF-EMF exposure may decrease the reproductive ability of males, but long-term exposures had no effect on reproductive ability. Down-regulation of ark gene in the exposed males suggests that the decrease in reproductive capacity may be induced by the effects of ELF-EMF exposure on spermatogenesis through the caspase pathway. QRT-PCR analysis confirmed that jra, ark and decay genes were down regulated in males exposed for 1 Generation (1G) and 72 h, which suggests that apoptosis may be inhibited in vivo. ELF-EMF exposure may have accelerated cell senescence, as suggested by the down-regulation of both cat and jra genes and the up-regulation of hsp22 gene. Up-regulation of totA and hsp22 genes during exposure suggests that exposed flies might induce an in vivo immune response to counter the adverse effects encountered during ELF-EMF exposure. Down-regulation of cat genes suggests that the partial oxidative protection system might be restrained, especially during short-term exposures. This study demonstrates the bioeffects of ELF-EMF exposure and provides evidence for understanding the in vivo mechanisms of ELF-EMF exposure on male D. melanogaster.

(E) Lupke M, Frahm J, Lantow M, Maercker C, Remondini D, Bersani F, Simkó M. Gene expression analysis of ELF-MF exposed human monocytes indicating the involvement of the alternative activation pathway. Biochim Biophys Acta. 1763(4):402-12, 2006. (GE)

This study focused on the cell activating capacity of extremely low frequency magnetic fields (ELF-MF) on human umbilical cord blood-derived monocytes. Our results confirm the previous findings of cell activating capacity of ELF-MF (1.0 mT) in human monocytes, which was detected as an increased ROS release. Furthermore, gene expression profiling (whole-genome cDNA array Human Unigene RZPD-2) was performed to achieve a comprehensive view of involved genes during the cell activation process after 45 min ELF-MF exposure. Our results indicate the alteration of 986 genes involved in metabolism, cellular physiological processes, signal transduction and immune response. Significant regulations could be analyzed for 5 genes (expression >2- or <0.5-fold): IL15RA (Interleukin 15 receptor, alpha chain), EPS15R (Epidermal growth factor receptor pathway substrate 15 - like 1), DNMT3A (Hypothetical protein MGC16121), DNMT3A (DNA (cytosine-5) methyltransferase 3 alpha), and one gene with no match to known genes, DKFZP586J1624. Real-time RT-PCR analysis of the kinetic of the expression of IL15RA, and IL10RA during 45 min ELF-MF exposure indicates the regulation of cell activation via the alternative pathway, whereas the delayed gene expression of FOS, IL2RA and the melatonin synthesizing enzyme HIOMT suggests the suppression of inflammatory processes. Accordingly, we suggest that ELF-MF activates human monocytes via the alternative pathway.

(E) Luukkonen J, Liimatainen A, Höytö A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced genotoxic effects in human SH-SY5Y neuroblastoma cells. PLoS One. 2011 Mar 23;6(3):e18021. (GT, IA)

BACKGROUND: Extremely low frequency (ELF) magnetic fields (MF) are generated by power lines and various electric appliances. They have been classified as possibly carcinogenic by the International Agency for Research on Cancer, but a mechanistic explanation for carcinogenic effects is lacking. A previous study in our laboratory showed that pre-exposure to ELF MF altered cancer-relevant cellular responses (cell cycle arrest, apoptosis) to menadione-induced DNA damage, but it did not include endpoints measuring actual genetic damage. In the present study, we examined whether pre-exposure to ELF MF affects chemically induced DNA damage level, DNA repair rate, or micronucleus frequency in human SH-SY5Y neuroblastoma cells. **METHODOLOGY/PRINCIPAL FINDINGS:** Exposure to 50 Hz MF was conducted at 100 μT for 24 hours, followed by chemical exposure for 3 hours. The chemicals used for inducing DNA damage and subsequent micronucleus formation were menadione and methyl methanesulphonate (MMS). Pre-treatment with MF enhanced menadione-induced DNA damage, DNA repair rate, and micronucleus formation in human SH-SY5Y neuroblastoma cells. Although the results with MMS indicated similar effects, the differences were not statistically significant. No effects were observed after MF exposure alone. **CONCLUSIONS:** The results confirm our previous findings showing that pre-exposure to MFs as low as 100 μT alters cellular responses to menadione, and show that increased genotoxicity results from such interaction. The present findings also indicate that complementary data at several chronological points may be critical for understanding the MF effects on DNA damage, repair, and post-repair integrity of the genome.

(E) Luukkonen J, Liimatainen A, Juutilainen J, Naarala J. Induction of genomic instability, oxidative processes, and mitochondrial activity by 50Hz magnetic fields in human SH-SY5Y neuroblastoma cells. Mutat Res. 760:33-41, 2014. (GT, OX, IA)

Epidemiological studies have suggested that exposure to 50Hz magnetic fields (MF) increases the risk of childhood leukemia, but there is no mechanistic explanation for carcinogenic effects. In two previous studies we have observed that a 24-h pre-exposure to MF alters cellular responses to menadione-induced DNA damage. The aim of this study was to investigate the cellular changes that must occur already during the first 24h of exposure to MF, and to explore whether the MF-induced changes in DNA damage response can lead to genomic instability in the progeny of the exposed cells. In order to answer these questions, human SH-SY5Y neuroblastoma cells were exposed to a 50-Hz, 100-μT MF for 24h, followed by 3-h exposure to menadione. The main finding was that MF exposure was associated with increased level of micronuclei, used as an indicator of induced genomic instability, at 8 and 15d after the exposures. Other delayed effects in MF-exposed cells included increased mitochondrial activity at 8d, and increased reactive oxygen species (ROS) production and lipid peroxidation at 15d after the exposures. Oxidative processes (ROS production, reduced glutathione level, and mitochondrial superoxide level) were affected by MF immediately after the exposure. In conclusion, the present results suggest that MF exposure disturbs oxidative balance immediately after the exposure, which might explain our previous findings on MF altered cellular responses to menadione-induced DNA damage. Persistently elevated levels of micronuclei were found in the progeny of MF-exposed cells, indicating induction of genomic instability.

(E) Ma Q, Deng P, Zhu G, Liu C, Zhang L, Zhou Z, Luo X, Li M, Zhong M, Yu Z, Chen C, Zhang Y. Extremely low-frequency electromagnetic fields affect transcript levels of

neuronal differentiation-related genes in embryonic neural stem cells. PLoS One. 2014 Mar 3;9(3):e90041. doi: 10.1371/journal.pone.0090041. eCollection 2014. (GE)

Previous studies have reported that extremely low-frequency electromagnetic fields (ELF-EMF) can affect the processes of brain development, but the underlying mechanism is largely unknown. The proliferation and differentiation of embryonic neural stem cells (eNSCs) is essential for brain development during the gestation period. To date, there is no report about the effects of ELF-EMF on eNSCs. In this paper, we studied the effects of ELF-EMF on the proliferation and differentiation of eNSCs. Primary cultured eNSCs were treated with 50 Hz ELF-EMF; various magnetic intensities and exposure times were applied. Our data showed that there was no significant change in cell proliferation, which was evaluated by cell viability (CCK-8 assay), DNA synthesis (Edu incorporation), average diameter of neurospheres, cell cycle distribution (flow cytometry) and transcript levels of cell cycle related genes (P53, P21 and GADD45 detected by real-time PCR). When eNSCs were induced to differentiation, real-time PCR results showed a down-regulation of Sox2 and up-regulation of Math1, Math3, Ngn1 and Tuj1 mRNA levels after 50 Hz ELF-EMF exposure (2 mT for 3 days), but the percentages of neurons (Tuj1 positive cells) and astrocytes (GFAP positive cells) were not altered when detected by immunofluorescence assay. Although cell proliferation and the percentages of neurons and astrocytes differentiated from eNSCs were not affected by 50 Hz ELF-EMF, the expression of genes regulating neuronal differentiation was altered. In conclusion, our results support that 50 Hz ELF-EMF induce molecular changes during eNSCs differentiation, which might be compensated by post-transcriptional mechanisms to support cellular homeostasis.

(E) Mairs RJ, Hughes K, Fitzsimmons S, Prise KM, Livingstone A, Wilson L, Baig N, Clark AM, Timpson A, Patel G, Folkard M, Angerson WJ, Boyd M. Microsatellite analysis for determination of the mutagenicity of extremely low-frequency electromagnetic fields and ionising radiation in vitro. Mutat Res. 626(1-2):34-41, 2007. (GT, IA)

Extremely low-frequency electromagnetic fields (ELF-EMF) have been reported to induce lesions in DNA and to enhance the mutagenicity of ionising radiation. However, the significance of these findings is uncertain because the determination of the carcinogenic potential of EMFs has largely been based on investigations of large chromosomal aberrations. Using a more sensitive method of detecting DNA damage involving microsatellite sequences, we observed that exposure of UVW human glioma cells to ELF-EMF alone at a field strength of 1 mT (50 Hz) for 12 h gave rise to 0.011 mutations/locus/cell. This was equivalent to a 3.75-fold increase in mutation induction compared with unexposed controls. Furthermore, ELF-EMF increased the mutagenic capacity of 0.3 and 3 Gy gamma-irradiation by factors of 2.6 and 2.75, respectively. These results suggest not only that ELF-EMF is mutagenic as a single agent but also that it can potentiate the mutagenicity of ionising radiation. Treatment with 0.3 Gy induced more than 10 times more mutations per unit dose than irradiation with 3 Gy, indicating hypermutability at low dose.

(E) Mariucci G, Villarini M, Moretti M, Taha E, Conte C, Minelli A, Aristei C, Ambrosini MV.
Brain DNA damage and 70-kDa heat shock protein expression in CD1 mice exposed to extremely low frequency magnetic fields. Int J Radiat Biol. 86(8):701-710, 2010. (GT, LE)

PURPOSE: The question of whether exposure to extremely low frequency magnetic fields (ELF-MF), may contribute to cerebral cancer and neurodegeneration is of current interest. In this study we investigated whether exposure to ELF-MF (50 Hz-1 mT) harms cerebral DNA and induces expression of 70-kDa heat shock protein (hsp70). **MATERIALS AND METHODS:** CD1 mice were exposed to a MF (50 Hz-1 mT) for 1 or 7 days (15 h/day) and sacrificed either at the end of exposure or after 24 h. Unexposed and sham-exposed mice were used as controls. Mouse brains were dissected into cerebral cortex-striatum, hippocampus and cerebellum to evaluate primary DNA damage and hsp70 gene expression. Food intake, weight gain, and motor activity were also evaluated. **RESULTS:** An increase in primary DNA damage was detected in all cerebral areas of the exposed mice sacrificed at the end of exposure, as compared to controls. DNA damage, as can be evaluated by the comet assay, appeared to be repaired in mice sacrificed 24 h after a 7-day exposure. Neither a short (15 h) nor long (7 days) MF-exposure induced hsp70 expression, metabolic and behavioural changes. **CONCLUSIONS:** These results indicate that in vivo ELF-MF induce reversible brain DNA damage while they do not elicit the stress response.

(E) Markkanen A, Juutilainen J, Naarala J. Pre-exposure to 50 Hz magnetic fields modifies menadione-induced DNA damage response in murine L929 cells. Int J Radiat Biol. 84(9):742-751, 2008. (IA)

PURPOSE: Effects on DNA damage response were investigated in murine L929 cells exposed to 50 Hz magnetic fields (MF) with or without ultraviolet B (UVB, wavelength 280-320 nm) radiation or menadione (MQ). **MATERIALS AND METHODS:** Cells were exposed to MF at 100 or 300 microT combined with MQ (150 microM, 1 hour) or UVB radiation (160 J/m(2)) using various exposure schedules. The samples were stained with propidium iodide (PI) and analysed by flow cytometer for cell cycle stages. Apoptotic cells were defined as sub G(1) events. **RESULTS:** In cells first exposed to 100 microT MF for 24 h, the response to subsequent MQ treatment was significantly altered so that the proportion of sub G(1) cells was decreased and the proportion of cells in the G(2)/M phase was increased. When a 300 microT MF was used, also the proportion of cells in the G(1) phase was decreased. MF exposures after MQ treatment did not alter responses to MQ. No effects were found from MF exposure alone or from MF combined with UVB radiation. **CONCLUSIONS:** The results strengthen previous findings suggesting that pre-exposure to MF can alter cellular responses to other agents, and indicate that MF as low as 100 microT has measurable impacts on cancer-relevant cellular processes such as DNA-damage.

(NE) Mizuno K, Narita E, Yamada M, Shinohara N, Miyakoshi J. ELF magnetic fields do not affect cell survival and DNA damage induced by ultraviolet B. Bioelectromagnetics. 35(2):108-115, 2014. (GT, IA)

We investigated whether extremely low frequency (ELF) magnetic field exposure has modification effects on cell survival after ultraviolet B (UV-B) irradiation and on repair process of DNA damage induced by UV-B irradiation in WI38VA13 subcloned 2RA and XP2OS(SV) cells. The ELF magnetic field exposure was conducted using a Helmholtz coil-based system that was designed to generate a sinusoidal magnetic field at 5 mT and 60 Hz. Cell survival was assessed by WST assay after UV-B irradiation at 20-80 J/m(2) , ELF magnetic field exposure for

24 h, followed by incubation for 48 h. DNA damage was assessed by quantification of cyclobutane pyrimidine dimer formation and 6-4 photoproduct formation using ELISA after UV-B irradiation at 20-80 J/m(2) followed by ELF magnetic field exposure for 24 h. No significant changes were observed in cell survival between ELF magnetic field and sham exposures. Similarly, DNA damage induced by UV-B irradiation did not change significantly following ELF magnetic field exposure. Our results suggest that ELF magnetic field exposure at 5 mT does not have modification effect on cell survival after UV-B irradiation and on repair process of DNA damage induced by UV-B irradiation.

(E) Nikolova T, Czyz J, Rolletschek A, Blyszczuk P, Fuchs J, Jovtchev G, Schuderer J, Kuster N, Wobus AM. Electromagnetic fields affect transcript levels of apoptosis-related genes in embryonic stem cell-derived neural progenitor cells. ASEB J 19(12):1686-1688, 2005. (GT, GE)

Mouse embryonic stem (ES) cells were used as an experimental model to study the effects of electromagnetic fields (EMF). ES-derived nestin-positive neural progenitor cells were exposed to extremely low frequency EMF simulating power line magnetic fields at 50 Hz (ELF-EMF) and to radiofrequency EMF simulating the Global System for Mobile Communication (GSM) signals at 1.71 GHz (RF-EMF). Following EMF exposure, cells were analyzed for transcript levels of cell cycle regulatory, apoptosis-related, and neural-specific genes and proteins; changes in proliferation; apoptosis; and cytogenetic effects. Quantitative RT-PCR analysis revealed that ELF-EMF exposure to ES-derived neural cells significantly affected transcript levels of the apoptosis-related bcl-2, bax, and cell cycle regulatory "growth arrest DNA damage inducible" GADD45 genes, whereas mRNA levels of neural-specific genes were not affected. RF-EMF exposure of neural progenitor cells resulted in down-regulation of neural-specific Nurr1 and in up-regulation of bax and GADD45 mRNA levels. Short-term RF-EMF exposure for 6 h, but not for 48 h, resulted in a low and transient increase of DNA double-strand breaks. No effects of ELF- and RF-EMF on mitochondrial function, nuclear apoptosis, cell proliferation, and chromosomal alterations were observed. We may conclude that EMF exposure of ES-derived neural progenitor cells transiently affects the transcript level of genes related to apoptosis and cell cycle control. However, these responses are not associated with detectable changes of cell physiology, suggesting compensatory mechanisms at the translational and posttranslational level.

(NE) Okudan N, Celik I, Salbacak A, Cicekcibasi AE, Buyukmumcu M, Gökbel H. Effects of long-term 50 Hz magnetic field exposure on the micro nucleated polychromatic erythrocyte and blood lymphocyte frequency and argyrophilic nucleolar organizer regions in lymphocytes of mice. Neuro Endocrinol Lett. 31(2):208-214, 2010. (GT)

OBJECTIVES: We aimed to investigate the effects of weak extremely low frequency electromagnetic fields (ELF-EMFs) on the nucleus size, the silver staining nucleolar organizer regions (AgNORs), the frequency of micro nucleated peripheral blood lymphocytes (MPBLs) and the micro nucleated polychromatic erythrocytes (MPCEs).**METHODS:** One hundred and twenty Swiss albino mice were equally divided into 6 groups. The study groups were exposed to 1, 2, 3, 4 and 5 microT 50 Hz-EMFs for 40 days. Micronucleus number (MN) per PBL was determined. **RESULTS:** ELF-EMF exposure caused a nonlinear decline of nucleus area. A sharp drop occurred in AgNOR area of 1 microT group, and following it gained an insignificantly higher level than that of the control group. The field did not change mean AgNOR numbers per nucleus of the groups. Relative AgNOR area had the highest level in 1 microT-exposure group, and the level was quite similar to that of the 5 microT-exposure group. The remaining groups had significantly lower values quite similar to that of the control level. The field exposure at any intensity did not affect significantly the frequency of either MPBLs or MPCEs. The number of MN per PBL in the 4 and 5 microT-exposure groups were significantly higher than those of the lower intensity exposure groups. The males in 4 microT-exposure group displayed the highest MN number per PBL, whereas values changed in a nonlinear manner. **CONCLUSIONS:** The results of the present study suggest that </=5 microT intensities of 50 Hz EMFs did not cause genotoxic effect on the mouse.

(E) Panagopoulos DJ, Karabarbounis A, Lioliousis C. ELF alternating magnetic field decreases reproduction by DNA damage induction. Cell Biochem Biophys. 67(2):703-16, 2013. (LE, GT, RP)

In the present experiments, the effect of 50-Hz alternating magnetic field on Drosophila melanogaster reproduction was studied. Newly eclosed insects were separated into identical groups of ten males and ten females and exposed to three different intensities of the ELF magnetic field (1, 11, and 21 G) continuously during the first 5 days of their adult lives. The reproductive capacity was assessed by the number of F1 pupae according to a well-defined protocol of ours. The magnetic field was found to decrease reproduction by up to 4.3%. The effect increased with increasing field intensities. The decline in reproductive capacity was found to be due to severe DNA damage (DNA fragmentation) and consequent cell death induction in the reproductive cells as determined by the TUNEL assay applied during early and mid-oogenesis (from germarium to stage 10) where physiological apoptosis does not occur. The increase in DNA damage was more significant than the corresponding decrease in reproductive capacity (up to ~7.5%). The TUNEL-positive signal denoting DNA fragmentation was observed exclusively at the two most sensitive developmental stages of oogenesis: the early and mid-oogenesis checkpoints (i.e. region 2a/2b of the germarium and stages 7-8 just before the onset of vitellogenesis)-in contrast to exposure to microwave radiation of earlier work of ours in which the DNA fragmentation was induced at all developmental stages of early and mid-oogenesis. Moreover, the TUNEL-positive signal was observed in all three types of egg chamber cells, mainly in the nurse and follicle cells and also in the oocyte, in agreement with the microwave exposure of our earlier works. According to previous reports, cell death induction in the oocyte was observed only in the case of microwave exposure and not after exposure to other stress factors as toxic chemicals or food deprivation. Now it is also observed for the first time after ELF magnetic field exposure. Finally, in contrast to microwave exposure of previous experiments of ours in which the germarium checkpoint was found to be more sensitive than stage 7-8, in the magnetic field exposure of the present experiments the mid-oogenesis checkpoint was found to be more sensitive than the germarium.

(E) Rageh MM, El-Gebaly RH, El-Bialy NS. Assessment of genotoxic and cytotoxic hazards in brain and bone marrow cells of newborn rats exposed to extremely low-frequency magnetic field. J Biomed Biotechnol. 2012;2012:716023. (LE, GT, DE, OX)

The present study aimed to evaluate the association between whole body exposure to extremely low frequency magnetic field (ELF-MF) and genotoxic , cytotoxic hazards in brain and bone marrow cells of newborn rats. Newborn rats (10 days after delivery) were exposed continuously to 50 Hz, 0.5 mT for 30 days. The control group was treated as the exposed one with the sole difference that the rats were not exposed to magnetic field. Comet assay was used to quantify the level of DNA damage in isolated brain cells. Also bone marrow cells were flushed out to assess micronucleus induction and mitotic index. Spectrophotometric methods were used to measure the level of malondialdehyde (MDA) and the activity of glutathione (GSH) and superoxide dismutase (SOD). The results showed a significant increase in the mean tail moment indicating DNA damage in exposed group ($P < 0.01, 0.001, 0.0001$). Moreover ELF-MF exposure induced a significant ($P < 0.01, 0.001$) four folds increase in the induction of micronucleus and about three folds increase in mitotic index ($P < 0.0001$). Additionally newborn rats exposed to ELF-MF showed significant higher levels of MDA and SOD ($P < 0.05$). Meanwhile ELF-MF failed to alter the activity of GSH. In conclusion, the present study suggests an association between DNA damage and ELF-MF exposure in newborn rats.

(E) Reyes-Guerrero G, Guzmán C, García DE, Camacho-Arroyo I, Vázquez-García M. Extremely low-frequency electromagnetic fields differentially regulate estrogen receptor-alpha and -beta expression in the rat olfactory bulb. Neurosci Lett. 471(2):109-13, 2010. (GE)

Recently, the effects of extremely low-frequency electromagnetic fields (ELF EMF) on biological systems have been extensively investigated. In this report, the influence of ELF EMF on olfactory bulb (OB) estrogen receptor-alpha (ER alpha) mRNA and -beta (ER beta) mRNA expression was studied by RT-PCR in adult female and male rats. Results reveal for the first time that ELF EMF exerted a biphasic effect on female OB ER beta mRNA gene expression, which increased during diestrous and decreased during estrous. We did not observe any influence of ELF EMF on female OB ER alpha mRNA expression. Our data demonstrate a fluctuating pattern of ER-alpha and -beta mRNA expression in the female OB throughout the phases of the estrous cycle in non-ELF EMF-exposed animals. Thus the highest ER alpha expression was observed in diestrous and the lowest in proestrous. The pattern of ER beta mRNA was less variable, the lowest expression was observed in diestrous. ER-alpha mRNA and -beta mRNA expression level in the male OB did not exhibit any variation either in ELF EMF-exposed or non-ELF EMF-exposed animals. In summary, ELF EMF modulate ER beta gene expression in the OB of female adult rats but not in males.

(E) Ruiz-Gómez MJ, Sendra-Portero F, Martínez-Morillo M. Effect of 2.45 mT sinusoidal 50 Hz magnetic field on Saccharomyces cerevisiae strains deficient in DNA strand breaks repair. Int J Radiat Biol. 86(7):602-611, 2010. (GT)

PURPOSE: To investigate whether extremely-low frequency magnetic field (MF) exposure produce alterations in the growth, cell cycle, survival and DNA damage of wild type (wt) and mutant yeast strains. **MATERIALS AND METHODS:** wt and high affinity DNA binding factor 1 (hdf1), radiation sensitive 52 (rad52), rad52 hdf1 mutant Saccharomyces cerevisiae strains were exposed to 2.45 mT, sinusoidal 50 Hz MF for 96 h. MF was generated by a pair of Helmholtz coils. During this time the growth was monitored by measuring the optical density at 600 nm and cell cycle evolution were analysed by microscopic morphological analysis. Then, yeast survival was assayed by the drop test and DNA was extracted and electrophoresed.

RESULTS: A significant increase in the growth was observed for rad52 strain (P = 0.005, Analysis of Variance [ANOVA]) and close to significance for rad52 hdf1 strain (P = 0.069, ANOVA). In addition, the surviving fraction values obtained for MF-exposed samples were in all cases less than for the controls, being the P value obtained for the whole set of MF-treated strains close to significance (P = 0.066, Student's t-test). In contrast, the cell cycle evolution and the DNA pattern obtained for wt and the mutant strains were not altered after exposure to MF. **CONCLUSIONS:** The data presented in the current report show that the applied MF (2.45 mT, sinusoidal 50 Hz, 96 h) induces alterations in the growth and survival of S. cerevisiae strains deficient in DNA strand breaks repair. In contrast, the MF treatment does not induce alterations in the cell cycle and does not cause DNA damage.

(E) Sarimov R, Alipov ED, Belyaev IY. Fifty hertz magnetic fields individually affect chromatin conformation in human lymphocytes: dependence on amplitude, temperature, and initial chromatin state. Bioelectromagnetics. 32(7):570-579, 2011. (GT)

Effects of magnetic field (MF) at 50 Hz on chromatin conformation were studied by the method of anomalous viscosity time dependence (AVTD) in human lymphocytes from two healthy donors. MF within the peak amplitude range of 5-20 μT affected chromatin conformation. These MF effects differed significantly between studied donors, and depended on magnetic flux density and initial condensation of chromatin. While the initial state of chromatin was rather stable in one donor during one calendar year of measurements, the initial condensation varied significantly in cells from another donor. Both this variation and the MF effect depended on temperature during exposure. Despite these variations, the general rule was that MF condensed the relaxed chromatin and relaxed the condensed chromatin. Thus, in this study we show that individual effects of 50 Hz MF exposure at peak amplitudes within the range of 5-20 μT may be observed in human lymphocytes in dependence on the initial state of chromatin and temperature.

(E) Tiwari R, Lakshmi NK, Bhargava SC, Ahuja YR. Epinephrine, DNA integrity and oxidative stress in workers exposed to extremely low-frequency electromagnetic fields (ELF-EMFs) at 132 kV substations. Electromagn Biol Med. 2014 Jan 24. [Epub ahead of print] (LE, GT, HU, OX)

There is apprehension about widespread use of electrical and electromagnetic gadgets which are supposed to emit electromagnetic radiations. Reports are controversy. These electromagnetic fields (EMFs) have considerable effect on endocrine system of exposed subjects. This study was focused to assess the possible bioeffects of extremely low-frequency (ELF)-EMFs on epinephrine level, DNA damage and oxidative stress in subjects occupationally exposed to 132 kV high-voltage substations. The blood sample of 142 exposed subjects and 151 non-exposed individuals was analyzed. Plasma epinephrine was measured by enzyme-linked immunosorbent assay, DNA damage was studied by alkaline comet assay along with oxidative stress. Epinephrine levels of sub-groups showed mean concentration of 75.22 ± 1.46, 64.43 ± 8.26 and 48.47 ± 4.97 for high, medium and low exposed groups, respectively. DNA damage ranged between 1.69 μm and 9.91 μm. The oxidative stress levels showed significant increase. The individuals employed in the live-line procedures were found to be vulnerable for EM stress with altered epinephrine concentrations, DNA damage and increased oxidative stress.

(E) Udroiu I, Cristaldi M, Ieradi LA, Bedini A, Giuliani L, Tanzarella C. Clastogenicity and aneuploidy in newborn and adult mice exposed to 50 Hz magnetic fields. Int J Radiat Biol. 82(8):561-567, 2006. (GT, DE, LE)

PURPOSE: To detect possible clastogenic and aneugenic properties of a 50 Hz, 650 muT magnetic field. **MATERIALS AND METHODS:** The micronucleus test with CREST (Calcinosis, Raynaud's phenomenon, Esophageal dismotility, Sclerodactility, Telangectasia) antibody staining was performed on liver and peripheral blood sampled from newborn mice exposed to an ELF (Extremely Low Frequency) magnetic field during the whole intra-uterine life (21 days), and on bone marrow and peripheral blood sampled from adult mice exposed to the same magnetic field for the same period. **RESULTS:** Data obtained in newborn mice show a significant increase in micronuclei frequencies. In absolute terms, most of the induced micronuclei were CREST-negative (i.e., formed by a chromosome fragment). However, in relative terms, ELF exposure caused a two-fold increase in CREST-negative micronuclei and a four-fold increase in CREST-positive micronuclei (i.e., formed by a whole chromosome). No significant effect was recorded on exposed adults. **CONCLUSIONS:** These findings suggest the need for investigation of aneugenic properties of ELF magnetic fields in order to establish a possible relationship to carcinogenesis.

(NE) Verschaeve L, Anthonissen R, Grudniewska M, Wudarski J, Gevaert L, Maes A. Genotoxicity investigation of ELF-magnetic fields in Salmonella typhimurium with the sensitive SOS-based VITOTOX test. Bioelectromagnetics. 32(7):580-584, 2011. (GT, IA)

We performed a genotoxicity investigation of extremely low-frequency (ELF) magnetic fields (MFs, 50 Hz, 100 and 500 µT, 1 and 2 h exposure) alone and in combination with known chemical mutagens using the VITOTOX test. This test is a very sensitive reporter assay of Salmonella typhimurium bacteria based on the SOS response. Our study showed that ELF-MFs do not induce SOS-based mutagenicity in S. typhimurium bacteria and do not show any synergetic effect when combined with chemical mutagens.

(E) Villarini M, Ambrosini MV, Moretti M, Dominici L, Taha E, Piobbico D, Gambelunghe C, Mariucci G. Brain hsp70 expression and DNA damage in mice exposed to extremely low frequency magnetic fields: a dose-response study. Int J Radiat Biol. 89(7):562-570, 2013. (LE, GT)

Purpose: To determine whether a dose-response relationship exists among exposure to extremely low frequency magnetic fields (ELF-MF) at different densities and 70-kDa heat shock protein (hsp70) expression and DNA damage in mouse brain. Materials and Methods: Male CD1 mice were exposed to ELF-MF (50 Hz; 0.1, 0.2, 1 or 2 mT) for 7 days (15 hours/day) and sacrificed either at the end of exposure or after 24 h. Hsp70 expression was determined in cerebral cortex-striatum, hippocampus and cerebellum by real-time reverse-transcriptase polymerase chain reaction (RT-PCR) and western blot analysis. Primary DNA damage was evaluated in the same tissues by comet assay. Sham-exposed mice were used as controls. Results: No changes in both hsp70 mRNA and corresponding protein occurred following exposure to ELF-MF, except for a weak increase in the mRNA in hippocampus of exposed mice to 0.1 mT ELF-MF. Only mice exposed to 1 or 2 mT and sacrificed immediately after exposure presented DNA strand breaks higher than controls in all the cerebral areas; such DNA breakage reverted to baseline in the mice sacrificed 24 h after exposure. Conclusions: These data show that high density ELF-MF only induce reversible brain DNA damage while they do not affect hsp70 expression.

(E) Wahab MA, Podd JV, Rapley BI, Rowland RE. Elevated sister chromatid exchange frequencies in dividing human peripheral blood lymphocytes exposed to 50 Hz magnetic fields. Bioelectromagnetics. 28(4):281-288, 2007. (GT, WS)

The in vitro cytomolecular technique, sister chromatid exchange (SCE), was applied to test the clastogenic potentiality of extremely low frequency (ELF) electromagnetic fields (EMFs) on human peripheral blood lymphocytes (HPBLs). SCE frequencies were scored in dividing peripheral blood lymphocytes (PBLs) from six healthy male blood donors in two rounds of experiments, R1 and R2, to determine reproducibility. Lymphocyte cultures in the eight experiments conducted in each round were exposed to 50 Hz sinusoidal (continuous or pulsed) or square (continuous or pulsed) MFs at field strengths of 1 microT or 1 mT for 72 h. A significant increase in the number of SCEs/cell in the grouped experimental conditions compared to the controls was observed in both rounds. The highest SCE frequency in R1 was 10.03 for a square continuous field, and 10.39 for a square continuous field was the second highest frequency in R2. DNA crosslinking at the replication fork is proposed as a model which could explain the mechanistic link between ELF EMF exposure and increased SCE frequency.

(E) Wang Z, Sarje A, Che PL, Yarema KJ. Moderate strength (0.23-0.28 T) static magnetic fields (SMF) modulate signaling and differentiation in human embryonic cells. BMC Genomics. 10:356, 2009. (GE)

BACKGROUND: Compelling evidence exists that magnetic fields modulate living systems. To date, however rigorous studies have focused on identifying the molecular-level biosensor (e.g., radical ion pairs or membranes) or on the behavior of whole animals leaving a gap in understanding how molecular effects are translated into tissue-wide and organism-level responses. This study begins to bridge this gulf by investigating static magnetic fields (SMF) through global mRNA profiling in human embryonic cells coupled with software analysis to identify the affected signaling pathways. **RESULTS:** Software analysis of gene expression in cells exposed to 0.23-0.28 T SMF showed that nine signaling networks responded to SMF; of these, detailed biochemical validation was performed for the network linked to the inflammatory cytokine IL-6. We found the short-term (<24 h) activation of IL-6 involved the coordinate up-regulation of toll-like receptor-4 (TLR4) with complementary changes to NEU3 and ST3GAL5 that reduced ganglioside GM3 in a manner that augmented the activation of TLR4 and IL-6. Loss of GM3 also provided a plausible mechanism for the attenuation of cellular responses to SMF that occurred over longer exposure periods. Finally, SMF-mediated responses were manifest at the cellular level as morphological changes and biochemical markers indicative of pre-oligodendrocyte differentiation. **CONCLUSION:** This study provides a framework describing how magnetic exposure is transduced from a plausible molecular biosensor (lipid membranes) to cell-level responses that include differentiation toward neural lineages. In addition, SMF provided a stimulus that uncovered new relationships - that exist even in the absence of magnetic fields - between gangliosides, the time-dependent regulation of IL-6 signaling by these glycosphingolipids, and the fate of embryonic cells.

(NE) Williams PA, Ingebretsen RJ, Dawson RJ. 14.6 mT ELF magnetic field exposure yields no DNA breaks in model system Salmonella, but provides evidence of heat stress protection. Bioelectromagnetics. 27(6):445-450, 2006. (GT)

In this study, we demonstrate that common extremely low frequency magnetic field (MF) exposure does not cause DNA breaks in this Salmonella test system. The data does, however, provide evidence that MF exposure induces protection from heat stress. Bacterial cultures were exposed to MF (14.6 mT 60 Hz field, cycled 5 min on, 10 min off for 4 h) and a temperature-matched control. Double- and single-stranded DNA breaks were assayed using a recombination event counter. After MF or control exposure they were grown on indicator plates from which recombination events can be quantified and the frequency of DNA strand breaks deduced. The effect of MF was also monitored using a recombination-deficient mutant (recA). The results showed no significant increase in recombination events and strand breaks due to MF. Evidence of heat stress protection was determined using a cell viability assay that compared the survival rates of MF exposed and control cells after the administration of a 10 min 53 degrees C heat stress. The control cells exhibited nine times more cell mortality than the MF exposed cells. This Salmonella system provides many mutants and genetic tools for further investigation of this phenomenon.

(E) Yokus B, Akdag MZ, Dasdag S, Cakir DU, Kizil M. Extremely low frequency magnetic fields cause oxidative DNA damage in rats. Int J Radiat Biol. 84(10):789-795, 2008. (GT)

PURPOSE: To detect the genotoxic effects of extremely low frequency (ELF) -magnetic fields (MF) on oxidative DNA base modifications [8-hydroxyguanine (8-OH-Gua), 2,6-diamino-4-hydroxy-5-formamidopyrimidine (FapyGua) and 4,6-diamino-5-formamidopyrimidine (FapyAde)] in rat leucocytes, measured following exposure to ELF-MF. **MATERIALS AND METHODS:** After exposure to ELF-MF (50 Hz, 100 and 500 microT, for 2 hours/day during 10 months), DNA was extracted, and measurement of DNA lesions was achieved by gas chromatography/mass spectrometry (GC/MS) and liquid chromatography/mass spectrometry (LC/MS). **RESULTS:** Levels of FapyAde, FapyGua and 8OHdG in DNA were increased by both 100 microT and 500 microT ELF-MF as compared to a cage-control and a sham group; however, statistical significance was observed only in the group exposed to 100 microT. **CONCLUSION:** This is the first study to report that ELF-MF exposure generates oxidatively induced DNA base modifications which are mutagenic in mammalian cells, such as FapyGua, FapyAde and 8-OH-Gua, in vivo. This may explain previous studies showing DNA damage and genomic instability. These findings support the hypothesis that chronic exposure to 50-Hz MF may be potentially genotoxic. However, the intensity of ELF-MF has an important influence on the extent of DNA damage.

(E) Yoon HE, Lee JS, Myung SH, Lee YS. Increased γ-H2AX by exposure to a 60-Hz magnetic fields combined with ionizing radiation, but not hydrogen peroxide, in non-tumorigenic human cell lines. Int J Radiat Biol. 2014 Jan 28. [Epub ahead of print] (GT, IA)

Purpose: Genotoxic effects have been considered the gold standard to determine if an environmental factor is a carcinogen, but the currently available data for extremely low frequency time-varying magnetic fields (ELF-MFs) remain controversial. As an environmental stimulus, the effect of ELF-MF on cellular DNA may be subtle. Therefore, a more sensitive method and systematic research strategy are warranted to evaluate genotoxicity. Materials and methods: We investigated the effect of ELF-MFs in combination with ionizing radiation (IR) or H_2O_2 on the DNA damage response of expression of phosphorylated H2AX (γ-H2AX) and production of γ-H2AX foci in non-tumorigenic human cell systems consisting of human lung fibroblast WI38 cells and human lung epithelial L132 cells. Results: Exposure to a 60-Hz, 2 mT ELF-MFs for 6 h produced increased γ-H2AX expression, as well as γ-H2AX foci production, a common DNA double-strand break (DSB) marker. However, exposure to a 1 mT ELF-MFs did not have the same effect. Moreover, 2 mT ELF-MFs exposure potentiated the expression of γ-H2AX and γ-H2AX foci production when combined with IR, but not when combined with H_2O_2. Conclusions: ELF-MFs could affect the DNA damage response and, in combination with different stimuli, provide different effects on γ-H2AX.

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 7

Evidence for Stress Response
(Stress Proteins)

Health Risk of Electromagnetic Fields: Research on the Stress Response

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A Scientific Perspective on Health Risk of Electromagnetic Fields: Research on the Stress Response

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I. Abstract

The stress response is a protective cellular mechanism that is characterized by stress protein synthesis. The stress response, by its very nature, shows that *cells react to EMFs as potentially harmful*. The stress response is an important protective mechanism that enables cells from animals, plants and bacteria to survive environmental stressors with the aid of heat shock proteins (hsp). It is stimulated by both non-thermal power (ELF), and non-thermal radiofrequency (RF) as well as thermal radio (RF) frequency EMFs, so the greatly differing energies are not critical in activating the DNA to synthesize proteins. Direct interaction of both ELF and RF EMFs with DNA is likely, since specific DNA sequences are sensitive to EMFs and retain their sensitivity when transferred to artificial molecular constructs. Basic science research is essential for determining the biological parameters needed to assess health risks of electromagnetic fields (EMFs) and the molecular mechanisms that explain them. However, the adversarial nature of the debate about risk has clouded the evaluation of the science. To clarify the results of research on EMF stimulation of the stress response, it is necessary to consider the scientific context as well as the research. There is ample evidence that ELF and RF fields activate DNA in cells and cause damage at exposure levels that are considered 'safe' (i.e., below current exposure limits that are based on tissue heating as measured in Specific Absorption Rate or SAR). Because non-thermal EMFs are biologically active and potentially harmful, new safety standards must be developed to protect against possible damage at non-thermal levels, and the standards must be defined in terms of a non-thermal biological dose. Fewer than one quarter of the relevant references listed in Table 1 appear in the IEEE list leading to the newly revised IEEE C95.1 recommendations (April, 2006).

II. Stress Proteins - Conclusions (Heat Shock Proteins)

Conclusion: *Scientific research has shown that the public is not being protected from potential damage that can be caused by exposure to EMF, both power frequency (ELF) and radio frequency (RF).*

Conclusion: *DNA damage (e.g., strand breaks), a cause of cancer, occurs at levels of ELF and RF that are below the safety limits. Also, there is no protection against cumulative effects stimulated by different parts of the EM spectrum.*

Conclusion: *The scientific basis for EMF safety limits is flawed when the same biological mechanisms are activated in ELF and RF ranges at vastly different levels of the Specific Absorption Rate (SAR). Activation of DNA to synthesize stress proteins (the stress response), is stimulated in the ELF at a non-thermal SAR level that is over a billion times lower than the same process activated in the RF at the thermal level.*

Conclusion: *There is a need for a biological standard to replace the thermal standard and to also protect against cumulative effects across the EM spectrum.*

III. ELF and RF activation of the stress response

Much detailed information about the stress response will be presented in the following sections and in the tables, but the most important finding to keep in mind is that *both ELF and RF fields activate the synthesis of stress proteins*. All cells do not respond to EMF, but activation of the same cellular mechanism by both thermal and non-thermal stimuli in a variety of cells shows that both ELF and RF are biologically active and that a biological 'dose' of EMF cannot be described in terms of SAR (Blank and Goodman, 2004a). SAR is irrelevant for non-thermal ELF responses, where energy thresholds are many orders of magnitude lower than in RF. A new definition of EMF dose is necessary for describing a safety limit, and SAR must be replaced by a measure of exposure that can be defined in biological terms.

The stress response, by its very nature, shows that *cells react to EMFs as potentially harmful*. The stress response is an important protective mechanism that enables cells from animals, plants and bacteria to survive environmental stressors, such as sharp increases in temperature (originally called 'heat shock'), hypoxia, and dissolved toxic heavy metals like Cd^{+2} and oxidative species that can damage proteins and DNA ('oxidative stress'). The stress response is evolutionarily conserved in essentially all eukaryotic and prokaryotic organisms, but not all stressors are effective in all cells, and different stress proteins are activated under different conditions. Stress proteins are a family of about 20 different proteins, ranging in size from a few kilodaltons to over 100kD. The 27kD and 70kD protein families are the most common and most frequently studied.

Kültz (2005) has called the stress response a '... defense reaction of cells to damage that environmental forces inflict on macromolecules.', based on evidence from gene analysis showing that the stress response is a reaction to molecular damage. The genes activated as a group along with stress genes, which Kültz calls the 'universally conserved proteome', are those associated with sensing and repairing damage to DNA and proteins. Stress proteins help damaged proteins refold to regain their conformations, and also act as "chaperones" for transporting cellular proteins to their destinations in cells. The molecular damage stimulated by non-thermal ELF fields occurs in the absence of an increase in temperature. ELF energy thresholds are estimated to be about 10^{-12} W/kg, over a billion times lower than the thermal stimuli that cause damage in the RF range (Blank and Goodman, 2004a).

The classic stress response to a sharp increase in temperature (i.e., 'heat shock') is associated with a biochemical pathway where transcription factors known as heat shock factors, HSFs, translocate from the cytoplasm to the nucleus, trimerize and bind to DNA at the heat shock elements (HSEs) in the promoters of the genes. The promoter is the DNA segment where protein synthesis is initiated and it is not part of the coding region.

The HSEs contain specific nucleotide sequences, nGAAn, that are the consensus sequences for thermal stimuli. The binding of HSFs to HSEs, etc is similar for heat shock in plant, animal and bacterial cells. ELF range EMFs have been shown to follow the same sequence of events in inducing stress response proteins in human cells, including breast (MCF7, HTB124), leukemia (HL60), epithelial cells, as well as E. coli and yeast cells.

Studies done with chick embryos and cells from *Drosophila* and *Sciara* salivary gland chromosomes have produced graphic evidence of the effects of EMF. In *Drosophila* and *Sciara* salivary gland chromosomes, EMF causes the formation of 'puff's, enlarged regions along the chromosome, at loci associated with activation of heat shock genes. This is followed by elevated concentrations of transcripts at the sites and eventually stress protein synthesis (Goodman and Blank, 1998). The changes in chromosome morphology are characteristic of the stress response to both EMF and elevated temperature. Chick embryos develop hearts that stop beating when the oxygen concentration is lowered, but that can be protected and kept beating if stress proteins have been induced by ELF fields (DiCarlo et al, 1998) and in the RF range (Shallom et al, 2002).

The cellular response pathways to EMF have been characterized in the ELF range (Goodman and Blank, 2002), and have been found to share some of the characteristics of heat shock stress, such as the movement of heat shock factor monomers from the cytoplasm to the nucleus. The biochemical mechanism that is activated, the MAPK signaling pathway, differs from the thermal pathway (Goodman and Blank, 2002), but is the same as the non-thermal pathway in the RF range (Leszczynski et al, 2002).

The HSP70 gene is activated within minutes in cells exposed to ELF fields (Lin et al, 1997), and is accompanied by the binding of HSFs to the specific nucleotide sites in the promoter of the gene. However, different segments of the DNA promoter function as HSEs. Research in the ELF range has shown that the promoter of the major stress protein, hsp70, has two domains that respond to two different physical stimuli, EMF and an increase in temperature (Lin et al, 1999). The stimulus-specific domains have different DNA sequences that cannot be interchanged. The ***DNA consensus sequences that respond to EMF are nCTCTn*** (Lin et al, 1997; 1999). These differ from the nGAAn consensus sequences for thermal stimuli. The existence of two different consensus sequences that respond to EMF and temperature increase, respectively, are molecular evidence of different pathways that respond to non-thermal and thermal stimuli.

In another series of experiments, a DNA sequence from the promoter of an EMF sensitive gene was included in a construct containing a reporter gene, either chloramphenicol amino transferase (CAT) or luciferase. In each case, the construct proved to be EMF sensitive and reacted when an ELF field was applied (Lin et al, 2001). The ability to transfer EMF sensitive DNA sequences that subsequently respond to an EMF is further evidence linking the cellular response to a DNA structure.

In heat shock, the stress response is activated when extracellular signals affect receptors in the plasma membrane. This probably does not happen with an EMF, which can easily penetrate throughout the cell and whose actions are therefore not limited to the membrane. One can transfer the EMF response by transferring the DNA consensus sequences (Lin et al, 2001), so it is likely that the activation mechanism involves direct EMF interaction with the DNA consensus sequences. The cell based signal transduction pathways of the heat shock response are involved in regulation of the EMF stimulated process, probably through the feedback control mechanisms that respond to the stress proteins synthesized or the mRNA concentrations that code for them (Lin et al, 1998).

Repeated induction of the stress response in a cell has been shown to induce cytoprotection, a reduced response associated with restimulation (Blank and Goodman, 1998). This is analogous to thermotolerance, the reduced response to an increase in temperature after an initial heat shock response. Experiments with developing chick embryos show similar habituation to repeated stimulation in the ELF range (DiCarlo et al, 2002). There are different effects of continuous and intermittent EMF exposures that show feedback control features in the EMF stimulated stress response (Lin et al, 1997). This autoregulatory reaction is an indication that the thermotolerance mechanism is inherent in the response to a single stimulus as well.

It has now been shown in many laboratories that RF also stimulates the cellular stress response and cells start to synthesize stress proteins in many different kinds of cells (e.g., Kwee et al, 2001; Shallom et al, 2002; Leszczynski et al, 2002; Weisbrot et al, 2004). Cotgreave (2005) included many cells that did not synthesize stress proteins in response to RF stimulation in his summary of data. The listings in Table 1 contain additional positive and negative results. It is quite clear that certain cell lines do not respond to EMF by synthesizing stress proteins. The reasons are not known, but the changes in cells in tissue culture and in cancer cells may render some of them unable to respond to EMF. In addition to mutations in cell lines, pre-exposure to ambient ELF and RF fields in the laboratory can also affect an ability to respond. What we can say in summary at this stage is that:

- the stress response has been demonstrated in many cells and linked to changes in the DNA and chromosomes.

- there are similarities in stress protein synthesis stimulated in the non-thermal ELF and thermal RF frequency ranges.

- the biochemical mechanism that is activated is the same non-thermal pathway in both ELF and RF, and is not associated with the thermal response.

IV. DNA activation mechanisms: EMFs and electrons

We think of DNA as a very stable polymer that stores and transmits genetic information from generation to generation. However, DNA must also come apart relatively easily to enable the continuous protein synthesis that is needed to sustain living cells. Usually, this process is started when specialized proteins called transcription factors bind to DNA.

However, both ELF and RF fields also stimulate DNA to start protein synthesis. EMF stimulation of stress protein synthesis indicates activation of DNA, even by relatively weak non-thermal ELF. This raises the possibility that EMF can cause other changes in DNA that interfere with the copying and repair processes in DNA, and that can lead to mutations and cancer.

Protein synthesis starts when the two chains of DNA come apart to make an mRNA copy of the amino acid code for a particular protein. This occurs at the specific DNA segment where the transcription factor binds, and in forming a bond changes the electron distribution. Since recent research has shown electron conduction in DNA (Wan et al, 1999; 2000; Ratner, 1999; Porath et al, 2000; Giese and Spichty, 2000), it is possible that EMF affects electron distribution and movement in DNA, and helps it to come apart to initiate protein synthesis, not unlike the action of a transcription factor. Charge transport through DNA depends on the DNA sequence (Shao et al, 2005), and there are reasons to believe that EMFs would cause the DNA to come apart at the EMF consensus sequence, nCTCTn (Blank and Goodman, 2002).

The ability of relatively small perturbations to stimulate DNA to initiate biosynthesis is consistent with larger perturbations that lead to DNA strand breaks. Several experimental studies have reported both single and double strand breaks in DNA and other chromosome damage after exposure to ELF fields (Lai and Singh, 1997a; Ivancsits et al, 2005, Diem et al, 2005; Winker et al, 2005). Ivancsits et al (2005) found DNA damage in fibroblasts, melanocytes and rat granulosa cells, but not in lymphocytes, monocytes and skeletal muscle cells. Single and double strand breaks and other DNA damage after exposure to RF fields have also been reported (Phillips et al, 1998; Sarimov et al, 2004; Lai and Singh, 2005).

The Ivancsits, Diem and Winker studies cited above are part of the REFLEX Project, a collaboration of twelve laboratories in seven countries of the European Union (REFLEX, 2004). The group found that both ELF and RF exposures, below the current safety limits, modified the expression of many genes and proteins. They also reported DNA damage (e.g., strand breaks, micronuclei, chromosomal damage) due to ELF fields at exposures of 35μT. Similar genotoxic effects were produced in fibroblasts, granulosa cells and HL60 cells by RF fields at SARs between 0.3 and 2W/kg. The expression and phosphorylation of the stress protein hsp27 was one of the many proteins affected.

The REFLEX Project Report (2004) is available on the internet and well worth consulting as a source of much information about the effects on cells *in vitro* due to the ELF and RF exposures we encounter in our environment. The Report has an introduction by Ross Adey, one of the last things he wrote, telling us about the importance of establishing "...essential exposure metrics ... based on mechanisms of field interactions in tissues". One needs a biological metric in order to characterize EMF exposure.

The possibility that EMFs could cause greater damage to DNA in the RF range and at longer exposures was demonstrated by Phillips et al (1998) who reported more DNA breaks when cells were exposed at higher SARs. They suggested that the rate at which

DNA damage can be repaired (or eliminated by apoptosis) is limited, and when the rate of damage at the higher SARs exceeds the repair rate, there is the possibility of retaining mutations and initiating carcinogenesis. Chow and Tung (2000) reported that exposure to a 50Hz magnetic field enhances DNA repair through the induction of DnaK/J synthesis. The eternal struggle in cells and organisms between the forces tending to break things down (catabolism) and those tending to build up and repair (anabolism) probably accounts for much of the variability one finds in experiments with cells as well as with people.

The changes in DNA initiated by ELF fields cannot be explained by thermal effects. Electric and magnetic fields interact with charges and magnetic dipoles, and fundamental mechanisms must ultimately be based on these interactions. From the data in Table 2, it is clear that relatively little energy is needed for effects on electron transfer (Blank and Goodman, 2002; 2004b; Blank, 2005). The low energies needed to perturb DNA in the ELF range suggest that the mechanism involves electrons, e.g., probably in the H-bonds that hold the two chains of DNA together. Electrons have very high charge to mass ratio and are most likely to be affected even by weak electric and magnetic fields.

There are many indications that electrons are involved in EMF reactions with DNA. In experiments that stimulate the stress response, the estimated force of ~10^{-21} newtons that activates DNA can move a free electron about the length of a H-bond (~.3nm) in 1ns. The calculated electron velocity is comparable to electron velocities measured in DNA (Wan et al, 1999; 2000), and is also expected if electrons move at the ~nanometer/picosecond flickering rate of protons in H-bonded networks (Fecko et al, 2003) that would be present at normally hydrated DNA sites. Electrons can tunnel nanometer distances in proteins (Gray and Winkler, 2003), and experiments have shown comparable electron movement in DNA (Wan et al, 1999; 2000). Electrons might be expected to move more readily from the CTCT bases in the consensus sequence, because of their low electron affinities. Finally, ELF fields have been shown to accelerate electron transfer in oxidation-reduction reactions (Blank and Soo, 1998; 2003).

The fact that the same non-thermal mechanism is activated in ELF and RF ranges emphasizes that it is not the total energy associated with the EMF that is critical, but rather the regular oscillations of the stimulating force. As already mentioned earlier, the energy associated with each wave (i.e., energy/cycle) is more or less independent of the frequency. If the same energy is needed to reach threshold in both ELF and RF, the many repetitions at the higher frequency cause the non-thermal threshold to be reached in a shorter time and the total energy absorbed over time to increase with frequency. Even in the ELF range, where SAR levels are very low, the stress response is activated by short exposures to fields of less than 1µT, while single and double strand breaks in DNA have been reported at longer exposures to higher field strengths ~0.1mT (Lai and Singh, 2005). The two mechanisms appear to be related in that breaks in DNA appear to result from free radical mechanisms that also involve electron transfer reactions (Lai and Singh, 1997b).

The reaction of EMFs with DNA differs from those listed in Table 2 in that they appear to occur with equal ease at the widely differing frequencies in ELF and RF ranges. The frequency dependence of a reaction provides information about how time constants of charge transfer processes are affected by fields, and the frequency responses of the few EMF sensitive biological systems that have been studied suggest that fields are most effective at frequencies that are close to the natural rhythms of the processes affected (Blank and Soo, 2001a; Blank and Goodman, 2004b; Blank, 2005). Frequency optima for the enzymes, Na,K-ATPase and cytochrome oxidase, differ by an order of magnitude with maximums at about 60Hz and 800Hz, respectively (Blank and Soo, 2001a), in both cases close to the observed frequency maximum of the enzyme reaction. The rate constant of the BZ reaction is about 250Hz, the frequency of the rate limiting step in a multi-step process with at least 10 sub-reactions (Blank and Soo, 2003).

The electrons in DNA that are affected by EMFs are probably not engaged in electron transfer reactions. They respond to frequencies that range from ELF to RF and are more likely to be tied to the wide frequency range of fluctuations than to the frequency of a particular reaction. The displacement of electrons in DNA would charge small groups of base pairs and lead to disaggregation forces overcoming H-bonds, separating the two chains and enabling transcription. Studies have shown that biopolymers can be made to disaggregate when the molecular charge is increased (Blank, 1994; Blank and Soo, 1987). This explanation would also apply to the effect of applied electric fields that also activate DNA. Electric fields exert a force on electrons, and have been shown to stimulate protein synthesis in HL60 cells (Blank et al, 1992), E coli (Laubitz et al, 2006) and muscle *in vivo* (Blank, 1995). The genes for the hsp70 stress protein are more likely to be activated since they have been shown to be 'bookmarked' on the DNA chain, that is, more exposed to externally applied forces (Xing et al, 2005).

The outline of a plausible mechanism to account for EMF activation of DNA through interaction with electrons has relied on evidence from many lines of research. This mechanism may or may not hold up under further testing, but the experimental facts it is based on have been verified. It has been clearly demonstrated that exposure of cells to non-thermal power and thermal radio frequency EMFs, at levels deemed to be safe for human exposure, activate DNA production of stress proteins and could increase the number of DNA breaks. There is ample experimental evidence to support the possibility of DNA damage at non-thermal levels of exposure, and the need for greater protection.

V. The critical role of scientific research

The connection between the results of scientific research and assessing EMF risk does not appear to be working well. We all agree that EMFs are unsafe at the level where they cause electrocution, and that we must protect against that possibility. We also agree that if other risks are associated with EMFs, we must identify them and determine the exposure levels at which they occur. This task requires that we define a biological dose of EMF, and that we obtain information about cellular mechanisms activated at different doses. As we have seen, the currently accepted measure of EMF dose, the specific absorption rate (SAR), is definitely not a measure of the effective biological dose when stress protein synthesis can be stimulated by SAR levels that differ by many orders of magnitude in the ELF and RF ranges (Blank and Goodman, 2004a). Yet, there is strong opposition to accepting the consequences of these experimental facts.

Regarding EMF mechanisms, we still have much to learn, but we know that the energy and field strength thresholds of many biological reactions are very low (Table 2). These findings indicate that safe exposure levels for the public should be substantially lowered, if only as a precautionary measure. Even when stated in vague terms, so as to require little more than lip service, a precautionary policy has not yet been recommended by the WHO. Thus, the two main problems of research on EMF risk, defining a biological dose and the desired level of exposure protection, remain to be solved.

Scientific research can contribute to defining a biological dose, but the desired level of exposure protection is a more complicated issue. Guidance for EMF policy on exposure protection has come primarily from epidemiology studies of health risks associated with power lines in the case of ELF, and cell phones in the case of RF. Basic research studies do not provide insight into the effects of long term exposures that are so important in determining risk, and they appear to have been used almost entirely to probe biochemical mechanisms that might underlie health risks identified in epidemiology studies. However, the research does overcome a basic weakness of epidemiology studies, an inability to determine a causal relation and to rule out effects of possible confounders. Epidemiology studies can correlate EMF exposure and health effects in human populations, and show quantitative dose-response relations, but it is only when coupled with basic research on molecular mechanisms that one can test and establish the scientific plausibility of effects of exposure. This scientific capability has become more important with recent advances in research on DNA, where mutations associated with initiation and promotion of cancer can be identified. EMF laboratory research has also played an indirect role in the practical aspects of risk by showing that:

- many biological systems are affected by EMFs,
- EMFs compete with intrinsic forces in a system, so effects can be variable,
- many frequencies are active,
- field strength and exposure duration thresholds are very low,
- molecular mechanisms at very low energies are plausible links to disease (e.g., effect on electron transfer rates linked to oxidative damage, DNA activation linked to abnormal biosynthesis and mutation).

Research on the stress response, a protective mechanism that involves activation of DNA and protein synthesis, was not included in previous scientific reviews prior to evaluating safety standards, and thus provides additional insights into EMF interactions (Blank and Goodman, 2004a). Activation of this protective mechanism by non-thermal as well as thermal EMF frequencies has demonstrated:

- the reality and importance of non-thermal effects of EMFs,

- that cells react to an EMF as potentially harmful,

- the same biological reaction to an EMF can be activated in more than one division of the EM spectrum,

- direct interaction of ELF and RF with DNA has been documented and both activate the synthesis of stress proteins,

- the biochemical pathway that is activated is the same pathway in both ELF and RF and it is non-thermal,

- thresholds triggering stress on biological systems occur at environment levels on the order of 0.5 to 1.0 µT for ELF,

- many lines of research now point to changes in DNA electron transfer as a plausible mechanism of action as a result of non-thermal ELF and RF.

Given these findings, the *specific absorption rate (SAR) is not the appropriate measure of biological threshold or dose*, and should not be used as a basis for a safety standard since it regulates against thermal effects only.

Cellular processes are unusually sensitive to non-thermal ELF frequency fields. The thresholds for a number of biological systems are shown in Table 2, and many are in the range of 0.5 to 1.0 µT, not very much higher than the usual environmental backgrounds of ~0.1µT. The low biological thresholds in the non-thermal ELF range undermine claims that an EMF must increase the temperature in order to cause changes in cells. They also show that many biochemical reactions can be affected by relatively low field strengths, similar to those in the environment. -Non-thermal ELF fields can also cause DNA damage, and therefore add to health and safety concerns.

In addition to very low thresholds, exposure durations do not have to be very long to be effective. Litovitz et al (1991, 1993), working with the enzyme ornithine decarboxylase, have shown a full response to an EMF when cells were exposed for only 10sec. This occurred with ELF sine waves or ELF modulated 915MHz sine waves. The exposure had to be continuous, since gaps in the sine wave resulted in a reduced response. Interference with the sine wave in the form of superimposed ELF noise also reduced the response (Mullins et al, 1998). The interfering effect of noise has been shown in the RF range by Lai and Singh (2005), who reported that noise interferes with the ability of an RF signal to cause breaks in DNA strands. The decreased effect when noise is added to a signal is yet another indication that EMF energy is not the critical factor in causing a response.

The finding that the stress response threshold can be stimulated in both ELF and RF frequency ranges appears to suggest that the threshold is independent of EMF energy. Energy increases with the frequency, so compared to an ELF energy of ~1a.u. (arbitrary unit of energy), the energy at RF is ~10^{11}a.u. Actually, it is the energy/cycle that is independent of frequency. A typical ELF cycle at 10^2Hz lasts 10^{-2}sec and a typical RF cycle at 10^{11}Hz lasts 10^{-11}sec. Because the energy is spread over a different number of cycles each second in the two ranges, the same value of $\sim 10^{-2}$ a.u./cycle applies to both ELF and RF ranges.

An early review of the stress response in the ELF range (Goodman and Blank, 1998) summarized basic findings, and a more recent review by Cotgreave (2005) has provided much additional information, primarily on the RF range. Table 1 summarizes both ELF and RF studies (mainly frequencies 50Hz, 60Hz, 900MHz, 1.8GHz) relevant to stimulation of DNA and stress protein synthesis in many different cells. The list is not exhaustive, but the citations represent the different frequencies and biological systems, as well as the diversity of results in the literature. As already noted by Cotgreave (2005), the stress response does not occur in reaction to EMFs in all cells. A paper by Jin et al (2000), to be discussed later, shows that even the same cell line can give opposite results in the same laboratory. The stress response is an important topic in its own right, but its importance for EMF research is that it offers insights into EMF interaction mechanisms in the stimulation of DNA. On the practical level, the stress response has shown the need to replace the SAR standard to take into account non-thermal biological effects.

Differences in experimental results shown in Table 1 are not uncommon when studying phenomena that are not as yet well understood, and this frequently gives rise to controversy. In EMF research, however, other factors have contributed to a controversial scientific atmosphere. The following sections on the scientific context, as well as a critique of the review by Cotgreave, will show how discussion of the stress response and the absence of discussion on related topics have compromised the evaluation of the science. The discussion of stress response stimulation in ELF and RF ranges together with ideas on DNA mechanisms, has important implications regarding EMF risk and safety.

VI. The troubling context of today's science

The need to include basic research findings in assessment of health risks is clear, but it is equally important to make sure that these findings are properly evaluated. No less an authority on science than Donald Kennedy (2006), the current Editor of *Science*, wrote "...how competitive the scientific enterprise has become, and the consequential incentive to push (or shred) the ethical envelope". He was referring primarily to the controversial religious/ political atmosphere over such issues as evolution, stem cell research, etc, but he could just as easily have included economic factors. In the following quote, editors of the *Journal of the American Medical Association* (JAMA 284:2203-2208, 2000) pointed out distortions in the proof of effectiveness of drugs in studies supported by the drug industry:

> "*There is a growing body of literature showing that faculty who have industry ties are more likely to report results that are favorable to a corporate sponsor, are more likely to conduct research that is of lower quality, and are less likely to*

disseminate their results to the scientific community".

Even *The Wall Street Journal* (Jan 9, 2007), which generally presents favorable views of business, had a front page article on the controversy over whether mycotoxins produced by molds are harmful, that was critical of scientist-business community connections. They pointed out that some scientific experts in the professional societies, who had issued statements minimizing harmful effects, had not disclosed their links to companies defending lawsuits in this area.

The connection between scientific expertise, the research that is done, and the source of support, has always been an ethical gray area, but the above examples and recent instances of experimental fraud have reinforced the impression that the ethical standards of scientists have deteriorated considerably. In our area of interest, insufficient attention has been paid to the influence the power and communication industries may be having on the research of those assessing EMF safety. At the Third International Standard Setting Seminar (October 2003) in Guilin, China, Prof. Henry Lai of the University of Washington summarized 179 cell phone studies showing that independent researchers were twice as likely to report biological effects due to RF in comparison to those funded by industry. This was very much in line with the earlier JAMA comment on the drug industry. Published reports have started to appear (Hardell et al, 2006; Huss et al, 2007) documenting the correlation of EMF research outcome with the source of support. Recognition of the phenomenon is a first step toward minimizing abuses, and one hopes that this information will eventually be factored into evaluation of the experimental results. I am not overly optimistic, since those who wish their influence to remain hidden can channel support through unaffiliated committees with non-committal names.

Science is a cooperative enterprise in the long run, but in day-to-day practice, there has always been competition among scientists for recognition and support. In EMF research, the atmosphere has become especially adversarial in the selection of participants and subjects to be covered in recent evaluations. Two important examples are the International Committee on Electromagnetic Safety (ICES) and IEEE sponsored symposium on "Reviews of Effects of RF Energy on Human Health" (BEMS Supplement 6, 2003), and the more recent WHO sponsored symposium "Sensitivity of Children to EMF Exposure" (BEMS Supplement 7, 2005). Both collections of papers appeared in *Bioelectromagnetics*, the journal of the primary research society in this scientific specialty, where publication carries a certain aura of authority in the field. Of course, one expects the highest of ethical standards, and the editor assured everyone that normal reviewing procedures, etc, had been followed. However, all that had come after the scope of the papers had been narrowly defined so that there was no coverage of recent research on the EMF stimulated stress response or stimulation of DNA to initiate protein synthesis. An older mind set pervaded the choice of the topics and the papers. That mind set appeared to be stuck in the belief that non-thermal EMF was biologically inert, that the nucleus was an impregnable structure that unlocked the genetic information in its DNA only at the time of cell division, etc. These two meetings took place only a few years ago, in a world of science where it had already been known for some time that biochemical signals are continuously changing DNA in cell nuclei and mitochondria, turning on protein synthesis, checking and repairing DNA itself, etc. Research on the stress response had even shown that DNA was unusually sensitive to EMF by finding responses in the non-thermal ELF range. One expects to find such papers in symposia organized by the Mobile Manufacturers Forum, but not in *Bioelectromagnetics*.

A science based evaluation process cannot limit its scope of interest so as to ignore a research area that is so central in biology today, and that is obviously affected by EMF. Information on the EMF stimulated stress response and stimulation of DNA to initiate protein synthesis must be an integral part of the evaluation process, and its omission in earlier evaluations compromised the scientific basis of those reviews and distorted their conclusions.

It is ironic that the review in *Bioelectromagnetics* Supplement 6 listed as its first guiding principle that "The RF safety standard should be based on science", essentially a reaffirmation of the IEEE guideline for the revision of C95.1-1991 safety standards. Scientific research is designed to answer questions, and answers do not come from deciding *a priori* that certain types of studies are not relevant or can be ignored because they have not been adequately proven in the eyes of the organizers. Scientific method is not democratic. The word 'proof' in 'scientific proof' is best understood in terms of its older meaning of 'test'. It does not rely on an adversarial 'weight of the evidence', where opposing results and arguments are presented and compared. Answers do not come from keeping a scoreboard of positive versus negative results and merely tallying the numbers to get a score. In scientific proof, number and weight do not count. It is hard to see how the review in *Bioelectromagnetics* Supplement 6 could reconcile its advocacy of science as a guiding principle with its subsequent endorsement of "the weight of evidence approach" to be used in their assessment.

We should be reminded that 'scientific proof' is not symmetric (Popper, 1959). One cannot prove that EMF is harmless no matter how many negative results one presents. One single reproducible (significant) harmful effect would outweigh all the negative results.

The above characteristics of science are generally acknowledged to be valid as abstract principles, but in EMF research, it has been quite common to list positive and negative findings and thereby imply equal weights. Table 1 is an alphabetical listing by first author of positive and negative findings, with the negative studies indicated as **NO** in bold. There is no scoreboard, since the studies are on many different systems, etc, and not of the same quality. The listing is not meant to be complete or to be scored, but rather to present the variety of biological systems studied in the different EMF ranges.
Negative studies play an important role in science, and there is good reason to publish them when they are failures to replicate earlier positive results. This can often lead to important clarifications of the effect, the technique, etc. However, negative studies are being used in another way. Although they cannot prove there is no positive effect, they do have an influence in the unscientific 'weight of evidence approach'. In epidemiology, where it is difficult to compare studies done under different conditions, it is common to make a table of the positive and negative results. The simple listing has the effect of a

tally, and the overall score substitutes for an evaluation. In any case, one can write that the evidence is 'not consistent', 'not convincing' or claims are 'unsubstantiated' and therefore 'unproven'. The same is true in experimental studies. Funds are generally not available for an independent study to track down the causes of the differences in results, so the contradictory results are juxtaposed and a draw is implied. This is a relatively cheap but effective way to neutralize or negate a positive study.

VII. Replication and failures to replicate experimental results

Independent replication of experiments is an essential criterion for acceptance of a result and one of the pillars of scientific proof. However, as we shall see below, it is very difficult to actually replicate a biological experiment. We need only remember the experience with the 'Henhouse' project run by the Office of Naval Research many years ago, when chicken eggs from different suppliers led to different effects of EMFs on chick embryo development.

While scientists generally shun replications, some failures to replicate have been analyzed and explained. The two discussed below had the earmarks of replications, but neither was. In one case, it was clearly shown by Jin et al (2000) that the investigators failed to use the precise cell type population of the original experiment. Jin et al obtained HL60 cells from the two different sources used in the papers with the contradictory results, and showed that the cells had very different growth characteristics, significantly different reactivities and reactions to EMFs. It appears that even different samples of the same cell line in the same laboratory can have different responses to EMFs. The changes that occur in tissue culture over time can result in very different responses to EMFs.

In another example, Utteridge et al (2002) published a paper in *Radiation Research* meant to test the positive results of an earlier study (Repacholi et al, 1997) that had shown a twofold increase in lymphoma in mice exposed to cell phones. They failed to replicate the findings, but even a cursory reading of the paper showed that the study was poorly designed and executed, and was definitely not a replication. They had used a different exposure regimen and had manually handled the animals, an added stress on the mice. The cancer rate in the control group was three times the rate of the earlier study, possibly due to the handling, making it almost impossible to find any effect of cell phone exposure. There were also unusual inconsistencies in the published data, such as listing the weights of animals that had died months earlier. It is hard to see how the paper passed peer review. The Utteridge study self-destructed, and the results of the Repacholi study are still looked upon as showing a relation between RF and cancer in an animal model. However, there were scientific casualties, the peer review process of the journal and the credibility of its editors.

It may be appropriate to mention that *Radiation Research*, a journal devoted to research with ionizing radiation frequencies, has published studies that almost exclusively show no EMF effects. A quick glance at Table 1 will show that many of the '**NO** effect' listings are published in that journal. It has even gone beyond the frequency range defined in its title and published 'negative' studies in the non-ionizing frequency range. The internet edition of *Microwave News* has an explanation for why this journal repeatedly publishes negative research and appears to have become so politicized on the EMF issue.

It is not unusual for scientists to deviate from an original experimental protocol when repeating an experiment. They generally view the deviations as improvements in technique. Readers who have not worked on that particular system are unlikely to focus on a small difference that does not appear to be significant. Yet, even a small difference may lead to a failed replication. Blank and Soo (2003) showed that EMF accelerated the Belousov-Zhabotinsky (BZ) reaction, which is the catalyzed oxidation of malonic acid. A subsequent study reported no effect of EMF on the BZ reaction (Sontag, 2006), in essence a failed replication. In the second study, the authors did not apply the field at the time the reactants were mixed, as in the original, but only after the reaction was well under way for about seven minutes. This time difference was critical for a reaction that responds to EMF. Other reactions had responded to EMF (Blank and Soo, 2001b; Blank, 2005) only when the field was applied at time zero, when the intrinsic chemical forces were relatively weak. The effect of EMF was even shown to vary inversely with the opposing chemical forces of an enzyme (Blank, 2005). After seven minutes, the BZ reaction was running at full speed and the applied ELF fields were not strong enough to overcome the built up chemical forces.

The above paragraph points up a critical factor often overlooked in EMF experiments. EMF is only one of the factors that can affect the rate of a biochemical reaction, and a relatively weak one in the ELF range. It appears that when an EMF accelerates charge movements associated with a reaction, the applied field competes with intrinsic forces, and the ability to see an effect of the applied EMF depends on minimizing the other forces in the system. It is obvious that an important strategy to minimize unwanted biological effects due to EMF is to maintain intrinsic forces at optimal (healthy) levels.

In the above mentioned experiments with the Na,K-ATPase (Blank, 2005), it was found that the effect of an applied electric or magnetic field varied inversely with the activity of the enzyme, which could be changed by changing ion concentrations, temperature, inhibitors, or by the normal aging of the preparation. The effect of intrinsic activity was also observed in other systems, electron transfer from cytochrome C to cytochrome oxidase (Blank and Soo, 1998), and in the effect of temperature on the oxidation of malonic acid (Blank and Soo, 2003). Since the effect of EMF in an experiment can vary depending on the other forces acting in the system, it is important to make sure that all relevant parameters are identified and controlled. Replication of biological experiments must ensure a comparable level of intrinsic biological activity before a perturbing EMF is applied. This is especially difficult with enzyme preparations as they age.

In studies of stress protein synthesis, many factors must be considered, but the choice of cells is particularly important. Not all cells respond to EMF, and the results of many experiments have suggested ideas about critical properties that are apt to determine the response and also affect the ability to replicate an experimental result.

A quick look at Table 1 shows that tissue culture cells are more likely to show '**NO** effect'. That is not really surprising. Cells in tissue culture have changed significantly to enable them to live indefinitely in the unnatural conditions of a flask in a laboratory, and the changes could have made them unresponsive to EMF. The same is true of the changes in cancer cells, although some (e.g., MCF7) have responded to EMF (e.g., Liburdy et al, 1993), and in one cell line, HL60, some samples respond to EMF and others do not (Jin et al, 2000). On the other hand, the study by Czyz et al (2004) found that p53-deficient embryonic stem cells showed an increased EMF response, but the wild type did not. It is obviously difficult to make generalizations about the necessary conditions for a response to EMF when there are so many variations, and cells can undergo changes in tissue culture.

Some insight into differences between cells has been obtained from a broad study of genotoxic effects in different kinds of cells (Ivancsits et al, 2005). They found no effects with lymphocytes, monocytes and skeletal muscle cells, but did find effects with fibroblasts, melanocytes and rat granulosa cells. Other studies (e.g., Lantow et al, 2006b; Simko et al, 2006) have also found that the blood elements, such as lymphocytes and monocytes are natural cells that have not responded. From an evolutionary point of view, it may be that mobile cells can easily move away from a stress and there is little selective advantage to develop the stress response. The lack of response by skeletal muscle cells is easier to explain (Blank, 1995). It is known that cells containing fast muscle fibers do not synthesize hsp70, while those with slow fibers do. This evolutionary development protects cells from over-reacting to the high temperatures reached in fast muscles during activity.

Other natural cells listed in Table 1, such as epithelial, endothelial and epidermal cells, fibroblasts, yeast, E coli, developing chick eggs, the cells of *Drosophila*, *Sciara* and C elegans, have all been shown to respond. While experiments with non-responding cells have provided little information, studies of the differences between responding and non-responding cells may be the best experimental strategy for studying the stress response mechanism. Proteomics appears to be an excellent tool for answering many of the questions about the molecular mechanisms that are activated (Leszczynski et al, 2004).

In studies of stress protein synthesis, the time course of a response must be determined. There is generally a rapid induction and a slower falloff of response, but the kinetics can be affected by many other conditions of the experiment. It is, therefore, important to look for stress proteins when they are apt to be present, and not before they have been synthesized or after the response has decayed. This may be the explanation for the inability of Cleary et al, (1997) to observe stress proteins twenty-four hours after exposure. Some additional cautions to be aware of in contemplating or evaluating a study. For example, different stresses elicit different responses, so it is important to determine which of the ~20 different stress proteins are synthesized. The most frequently studied stress proteins are hsp70 and hsp27, but others may be involved and undetected. The exposure history of a cell population must be known, since there are differences in the responses to an initial stimulus and subsequent ones. The need to provide shielding for cells becomes far more complicated when they respond to RF as well as ELF fields and one must insure no pre-exposure.

Obviously, many experiments must be done to determine the optimal conditions for the study of a particular system. This does not shift the burden of proof to those unable to find an effect, but it adds weight to the cautions generally voiced in papers that state their failure to observe stress proteins 'under our experimental conditions'. Those words mean just that, and not that stress proteins were absent.

An experiment on EMF stimulation of cell growth that has almost disappeared from the EMF literature is the work of Robert Liburdy (Liburdy et al, 1993). He reported that weak 60Hz fields can interfere with the ability to inhibit growth in MCF7 breast cancer cells. This finding has been replicated six times, but the original experiment and its replications have been ignored by many health oriented scientists (Liburdy, 2003), including the recent WHO review (BEMS Supplement 7, 2005). Even breast cancer researchers (e.g., Loberg et al, 1999), who have not been directly involved in the EMF debate, appear to be totally unaware of results showing the ability of weak 60Hz fields to affect cancer cell growth. It is shocking when an EMF research review by a presumably scientifically neutral WHO fails to even mention any of the papers that offers insight into the mechanism of a devastating disease that is so prevalent in the population (Blank and Goodman, 2006). Let us not forget the asymmetry in scientific proof (Popper, 1959), where a single reproducible harmful effect would outweigh all the negative results. The many replications of the Liburdy experiment have given us a crucial finding regarding the question of EMF risk, and they cannot be ignored.

VIII. A critical look at a recent review of the stress response

The earlier discussion of non-scientific influences in the design and presentation of the results of EMF research serves as an introduction to a critical look at the recent review on RF and the stress response by Cotgreave (2005) 'with contributions of the Forschunggemeinschaft Funk'. I agree with the major conclusion-of the review, the need for more research on the stress response with better controls. However, Cotgreave was highly selective in his omission of papers on ELF and stress proteins. Given that there are many relevant ELF papers reporting effects on stress proteins at non-thermal levels, this omission results in significant under-reporting of what is scientifically established. These obvious and scientifically questionable omissions were used to cast doubt on the ability of RF to have a significant biological effect, at a time when much evidence pointed in the opposite direction.

Cotgreave stated correctly that RF is pleiotropic (produces more than one gene effect) for many regulatory events, in addition to the stress response. That observation comes as no surprise to biologists who know that cellular systems are interconnected and that the complexity of the signaling pathways resembles that of the old interlinked intermediary metabolism charts. It is also no surprise to those familiar with early papers on EMFs, which showed activation of genes such as c-*myc* (Goodman and Shirley-Henderson, 1991; Lin et al, 1994;1996) and c-*fos* (Rao and Henderson, 1996) at about the same time the EMF stress response was first described (Blank et al, 1994; Goodman et al, 1994). The EMF stimulated synthesis of many proteins (Goodman and Henderson, 1988) and the binding of specific transcription factors AP-1, AP-2 and SP-1 were also previously described (Lin et al, 1998).

By highlighting the previously known pleiotropic nature of the EMF response, Cotgreave played down the role of the stress response as a protective mechanism. Had he analyzed the biological implications of the many genes activated, he could have pointed to evidence from proteomics and gene analysis that there is a relevant pattern to the pleiotropism. Kültz (2005) recently summarized the evidence that specific groups of genes are activated along with stress genes across the biological spectrum. It is of particular interest to the EMF discussion that this 'universally conserved proteome' consists largely of genes involved in sensing and repairing damage to DNA and proteins, evidence that the stress response is a reaction to molecular damage across the biological spectrum. The stress response is one of many stimulated by RF, but other parts of the response also show evidence of damage control in reaction to an EMF.

By limiting the scope of his review to effects of RF, Cotgreave overlooked much that is relevant to understanding the effects of EMFs. That was a bit like writing a review on the physiological effects of alcohol and limiting the discussion to scotch whiskey. The EM spectrum is continuous and its divisions arbitrary, so there is no good reason to limit the discussion to RF when living cells are activated and synthesize stress proteins in both RF and ELF ranges (Blank and Goodman, 2004a). Furthermore, emissions from cell phones include both RF and ELF frequencies (Linde and Mild, 1997; Jokela, 2004; Sage et al, 2007). The bulk of the original research on EMFs and the stress response was done using ELF (see review by Goodman and Blank, 1998). ELF studies also led to information about the DNA consensus sequence sensitive to EMFs that differs from the 'heat shock' consensus sequence (Lin et al, 1999). This is a critical piece of molecular evidence showing the difference between thermal and non-thermal responses. Cotgreave described the heat shock consensus sequence, but not the EMF consensus sequence or the experiments in which such sequences were transferred and retained sensitivity to an EMF (Lin et al, 2001). For any insight into EMF-DNA interaction, it was absolutely essential to describe the molecularly based biological sensitivity to EMFs, inherent in DNA structure, that differs from thermal sensitivity and that can be manipulated.

More importantly, by considering both ELF and RF responses, it becomes obvious that the practice of describing EMF 'dose' in terms of SAR is meaningless for the stress response (Blank and Goodman, 2004a). The research on ELF stimulated stress response has shown unequivocally that SAR at the threshold is many orders of magnitude lower than in the RF range. The separation of thermal and non-thermal mechanisms had already been shown by Mashevich et al (2002), where chromosomal damage observed under RF in lymphocytes was not seen when the cells were exposed to elevated temperatures. The importance of non-thermal mechanisms was also made clear in the experiments of Bohr and Bohr (2000) in a much simpler biochemical system, showing that both denaturation and renaturation of β-lactoglobulin are accelerated by microwave EMF, and by de Pomerai et al (2003), who showed that microwave radiation causes protein aggregation without bulk heating. These as well as the ELF enzyme kinetics studies listed in Table 2 should have indicated that EMFs can cause changes in molecular structure without requiring heating.

Cotgreave overlooked a similarity between electric and magnetic ELF stimulation of DNA and endogenous electric stimulation of protein synthesis. Blank (1995) had reviewed this effect in striated muscle, and recently Laubitz et al (2006) showed that myoelectrical activity in the gut can trigger heat shock response in E coli and Caco-2 cells. The mechanism in striated muscle is well known. Body builders stimulate muscle activity to increase muscle mass, and biologists have known that the electric fields associated with muscle action potentials stimulate the synthesis of muscle proteins. The particular proteins synthesized appear to be related to the frequency of the action potentials, and one can even change the protein composition of a muscle by changing the frequency of the action potentials (Pette and Vrbova, 1992). Under normal physiological conditions, the action potentials along the muscle membrane drive currents across the DNA in nuclei adjacent to the membrane. The estimated magnitude of electric field, ~10V/m, provides a large safety margin in muscle, since fields as low as 3mV/m stimulate biosynthesis in HL60 cells (Blank et al, 1992). The fact that a physiological mechanism links electric stimulation to protein synthesis suggests that EMF can cause stress protein synthesis by a similar mechanism.

As a matter of proper scholarly attribution “heat shock’ was first described in Drosophila by Ritossa (1962), and the first description of stress response due to EMF was in back-to-back papers showing similar protein distributions stimulated by temperature and ELF (Blank et al, 1994), and that both stimuli resulted in proteins that reacted with the same specific antibody for the stress protein hsp70 (Goodman et al, 1994). The ability of power frequency fields to alter RNA transcription patterns had been reported even earlier by Goodman et al (1983).

The above discussion acknowledges that Cotgreave’s review was a positive contribution that summarized much useful information, but one that failed to properly assess the state of knowledge in EMF stress protein research. He gave the impression that much of the information was tenuous and that the thermal mechanism was the only one to consider. This may be his point of view and that of co-contributor, Forschunggemeinschaft Funk. However, at the very least, he should have incorporated relevant research on stimulation of the stress response by non-thermal EMFs. The ELF data have convinced many to reject the paradigm of thermal effects only. A reader would have learned more about the stress response had the author devoted more space to the ELF papers than to papers on something called ‘athermal heating’.

IX. Rethinking EMF safety in a biology context

Studies of the stress response in different cells under various conditions have enabled us to characterize the molecular mechanisms by which cells respond to EMF and their effects on health risk. That information can now correct assumptions about biological effects of EMF, and establish a scientific basis for new safety standards.

In setting standards, it is essential that basic findings in all relevant research areas are taken into account. Relevance is not subjective. It is determined by whether a study adds to our knowledge of how cells react to EMF, and this criterion determined inclusion of the references in Table 1. The criteria for the references in the IEEE list were not focused on the molecular biology of cellular responses that illuminate disease mechanisms, but were based on such assumptions as arbitrarily defined divisions of the spectrum, on thermal responses only, etc. It is therefore not surprising that many relevant studies were omitted in the IEEE literature review. Fewer than one quarter of the references listed in Table 1 appear in the IEEE list. The result of having omitted many EMF studies, including those on the stress response, is that many research results have not been utilized in setting EMF safety standards. A careful examination of basic assumptions will show that the omissions are crucial and that they indicate an urgent need to reconsider the entire basis for EMF safety standards. Here in bold are the assumptions, followed by the re-evaluations:

- **Safety standards are set by division of the EM spectrum.** It may come as a surprise to the engineers and physicists who set up the divisions of the EM spectrum, but biology does not recognize EM spectrum divisions. The same biological reaction can be stimulated in more than one subdivision of the EM spectrum. The arbitrarily defined divisions of the spectrum do not in any way confine the reactions of cells to EMF, and ELF studies do indeed contribute to an understanding of how cells respond to RF. This was discussed in the critique of Cotgreave's (2005) review. This area clearly demands immediate attention. People are getting ELF and RF simultaneously from the same device, and they are being protected from thermal effects only. This ignores the potentially harmful effects from non-thermal ELF and RF discussed next.

- **EMF standards are based on the assumption that only ionizing radiation causes chemical change.** The stress response in both ELF and RF ranges has shown that non-ionizing radiation also causes chemical change. Several additional examples of EMF stimulated chemical change in the ELF range are listed in Table 2.

- **EMF standards are based on the assumption that non-ionizing EMF only causes damage by heating (i.e., damage by thermal effects only).** Research on the stress response in the ELF range has shown that a thermal response to a rise in temperature and the non-thermal response to EMF are associated with different DNA segments of the same gene. Both the thermal and the non-thermal mechanisms are natural responses to potential damage.

Furthermore, the non-thermal stress response can occur in both the ELF and RF ranges. Other non-thermal effects of EMF have been demonstrated, e.g., acceleration of electron transfer reactions and DNA strand breaks.

- **Safety limits in the non-ionizing range are in terms of rate of heating (SAR).** The above described effects occur below the thermal safety limits in the non-ionizing range, so the safety limits provide no protection against non-thermal damage. Safety limits must include non-thermal effects.

X. Summary

It is generally agreed that EMF safety standards should be based on science, yet recent EMF research has shown that a basic assumption used to determine EMF safety is not valid. The safety standard assumes that EMF causes biological damage only by heating, but cell damage occurs in the absence of heating and well below the safety limits. This has been shown in the many studies, including the cellular stress response where cells synthesize stress proteins in reaction to potentially harmful stimuli in the environment, including EMF. The stress response to both the power (ELF) and radio (RF) frequency ranges shows the inadequacy of the thermal (SAR) standard.

The same mechanism is stimulated in both ranges, but in the ELF range, where no heating occurs, the energy input rate is over a billion times lower than in the RF range.

The stress response is a natural defense mechanism activated by molecular damage caused by environmental forces. The response involves activation of DNA, i.e., stimulating stress genes as well as genes that sense and repair damage to DNA and proteins. Scientific research has identified specific segments of DNA that respond to EMF and it has been possible to move these specific segments of DNA and transfer the sensitivity to EMF. At high EMF intensities, the interaction with DNA can lead to DNA strand breaks that could result in mutation, an initiating step in the development of cancer.

Scientific research has shown that ELF/RF interact with DNA to stimulate protein synthesis, and at higher intensities to cause DNA damage. The biological thresholds (field strength, duration) are well below current safety limits. To be in line with EMF research, a biological standard must replace the thermal (SAR) standard, which is fundamentally flawed. EMF research also indicates a need for protection against the cumulative biological effects stimulated by EMF across the EM spectrum.

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Table 1. Studies of EMF Stimulation of DNA and Protein Synthesis

(page 1)

Table 1 summarizes both ELF and RF studies (mainly frequencies 50Hz, 60Hz, 900MHz, 1.8GHz) relevant to stimulation of DNA and stress protein synthesis in many different cells.

Study/Journal	Frequency	Cells/effect on hsps
Balcer-Kubicek et al, 1996 Radiation Res	60Hz	HL60 **NO** synthesis of myc
Blank et al, 1994 Bioelectrochem Bioenerg	60Hz	*Sciara* salivary glands [temperature, EMF, cause same new proteins]
Capri et al, 2004 Int J Radiat Biol	1800MHz	monocytes **NO** effect on apoptosis, hsp70
Caraglia et al, 2005 J Cell Physiol	1.95GHz	epidermoid cancer cells Induces apoptosis, hsp70
Chauhan et al, 2006 Radiation Res	1.9GHz	human lymphoblastoma (TK6) **NO** hsp response
Chauhan et al, 2006 Int J Radiat Biol	1.9GHz	two human immune cell-lines HL60,MM6 **NO** hsp response
Cleary et al, 1997 Bioelectromagnetics	27MHz	HeLa, CHO (also at 2450MHz mammalian cells **NO** hsp after 2 hr exposure, 24 hr to measurement
Chow and Tung, 2000 FEBS Letters	50Hz	E. coli strain XL-1 BLUE + plasmid pUCB DNA repair improved
Czyz et al, 2004 Bioelectromagnetics	modulated 1.71GHz	p53-deficient embryonic stem cells hsp70 expression, but not in wild type

Table 1. Studies of EMF Stimulation of DNA and Protein Synthesis
(page 2)

Daniells et al, 1998	750MHz	C elegans
Mutat Res		induced hsp16
Dawe et al, 2005	750MHz	C elegans (same lab as above paper)
Bioelectromagnetics		hsp 16 may be due to temperature rise
Di Carlo et al, 2002	60Hz	chick embryo
J Cell Biochem		repeated EMF causes lower hsp response
Diem et al, 2005.	1800MHz	fibroblasts, GFSH-R-17 granulosa cells
Mutation Res		non-thermal DNA breakage
Fritze et al, 1997	900MHz	rat brain
Neuroscience		blood brain barrier leakage at high SAR
Goodman et al, 1983	pulsed 60Hz	*Sciara* larvae
Science		induce cellular transcription
Goodman et al, 1994	60Hz	*Sciara* larvae
Bioelectrochem Bioenerg		increased hsp70 transcripts
Harvey et al, 2000	864.3MHz	human mast cell line, HMC-1
Cell Biol Int		effects on protein kinase C , stress genes
Hirose et al, 2006a	2.1425GHz	Human IMR-90 fibroblasts
Bioelectromagnetics		**NO** effect on gene expression of p53
Hirose et al, 2006b	2.1425GHz	human glioblastoma A172, IMR-90
Bioelectromagnetics		fibroblasts
		NO effect on apoptosis,
		phosphorylation of hsp27
Ivancsits et al, 2005	intermittent 50Hz	**NO** effect lymphocyte, monocyte,
Mutation Res		muscle: DNA damage: fibroblast,
		melanocyte, rat granulose
Jin et al, 1997	60Hz	HL60 cells from two sources
Bioelectrochem Bioenerg		*myc* expression in one population, not in other
Kwee et al, 2001	960MHz	human epithelial amnion (AMA) cells
Electro- and Magnetobiology		hsp70 increased

Table 1. Studies of EMF Stimulation of DNA and Protein Synthesis
(page 3)

Lacy-Hulbert et al, 1995 Radiation Res	50Hz	HL60 **NO** synthesis of myc or β-actin
Lai & Singh, 1997a Bioelectromagnetics	60Hz	rat brain cells melatonin blocks DNA strand breaks
Lai & Singh, 2005 Electromag Biol Med	1800MHz	rat brain cells noise blocks DNA strand breaks
Lantow et al, 2006a Radiation Res	1800MHz	human Mono Mac 6 and K562 cells **NO** hsp response
Lantow et al, 2006b Radiat Environ Biophys	1800MHz	primary human monocytes, lymphocytes **NO** hsp response
Lantow et al, 2006c Radiation Res	1800MHz	human Mono Mac 6 and K562 cells **NO** effect on apoptosis or necrosis
Laszlo et al, 2005 Radiation Res	835MHz	cultured mammalian cells **NO** 'effect within sensitivity of assay'
Laubitz et al, 2006 Experimental Physiol	muscle generated ELF	E coli, Caco-2 cells induce hsp70, protect vs apoptosis
Lee JS et al, 2005 Int J Radiat Biol	849, 1763 MHz	hsp70.1-deficient mice **NO** hsp induction
Lee S et al, 2005 FEBS Lett	2.45GHz	cultured human cells gene regulation: apoptosis 88, cell cycle99
Leszczynski et al, 2002 Differentiation	900MHz	human endothelial cells activate hsp27/p38MAPK stress pathway
Liburdy et al, 1993 J Pineal Res	60Hz	ER^+ MCF7 breast cancer cells block melatonin's oncostatic action
Lim et al, 2005 Radiation Res	900MHz	human leukocytes. **NO** effect on hsp
Lin et al, 1994 J Cell Biochem	60Hz	human HL60 cells EMF region of the c-*myc* promoter

Table 1. Studies of EMF Stimulation of DNA and Protein Synthesis
(page 4)

Lin et al, 1996 Bioelectrochem Bioenerg	60Hz	human HL60 cells changes in c-myc transcript levels
Lin et al, 1999 J Cell Biochem	60Hz	human HL60 cells EMF consensus sequence in HSP70 promoter
Lin et al, 2001 J Cell Biochem	60Hz	human HL60 cells EMF consensus sequence response elements
Lixia et al, 2006 Mutat Res	1.8GHz	human lens epithelial cells increased hsp70 protein
Maes et al, 2006 [Epub] Mutagenesis	900MHz	peripheral blood lymphocytes **NO** effect on DNA damage
Malagoli et al, 2004 Comp Biochem Physiol	50Hz	mussel immunocyte activate p38 MAP kinase, induce hsp70, hsp90
Mashevich et al, 2003 Bioelectromagnetics	830MHz	human peripheral blood lymphocytes chromosomal instability
McNamee et al, 2002 Radiat Res	1.9Ghz	human leukocytes **NO** effect on DNA damage, micronuclei
Miyakawa et al, 2001 Bioelectromagnetics	60Hz	C elegans induction of hsp16
Nylund & Leszczynski,2004 Proteomics	900MHZ	human endothelial cell line EA.hy926 effects on cytoskeletal proteins
Nylund & Leszczynski,2006 Proteomics	900MHZ	human endothelial cell line EA.hy926 response genome- and proteome-dependent
Oktem et al, 2005. Arch Med Res	900MHz	rats (oxidative kidney damage) oxidative damage protected by melatonin
Ozguner et al, 2005 Toxicol Ind Health	900MHz	rats (oxidative myocardial damage) protection by caffeic acid phenethyl ester

Table 1. Studies of EMF Stimulation of DNA and Protein Synthesis

(page 5)

Penafiel et al, 1997 Bioelectromagnetics	840MHz (AM, FM)	mouse L929 cells (ornithine decarboxylase activity) frequency dependent AM effect, no FM effect
Phillips et al, 1998 Bioelectrochem Bioenerg	813, 836MHz	Molt-4 T-lymphoblastoid cells DNA damage (and ability to repair) varied with SAR
Saffer & Thurston, 1995 Radiation Res	60Hz	HL60, Daudi cells **NO** synthesis of myc
Sanchez et al, 2006 FEBS J	900MHz	human skin cells slight but significant increase in hsp70
Sarimov et al, 2004 IEEE Trans Plasma Sci	895, 915MHz	transformed human lymphocytes affect chromatin conformation
Shallom et al, 2002 J Cell Biochem	915MHz	chick embryos induces hsp70, protects against hypoxia
Shi et al, 2003. Environ health Perspect	60Hz	human keratinocytes **NO** phosphorylation, expression of hsp27
Simko et al, 2006 Toxicol Lett	900MHz	human Mono Mac 6 cells **NO** hsp reponse
Vanderwaal et al, 2006 \Int J Hyperthermia	900MHz	cultured HeLa, S3 and EA Hy296 cells **NO** hsp27 phosphorylation increases
Velizarov et al, 1999 Bioelectrochem Bioenerg	960MHz	human epithelial cells cell proliferation
Wang et al, 2006 Bioelectromagnetics	2450MHz	human glioma A172 cells **NO** hsp70, hsp27
Weisbrot et al, 2003 J Cell Biochem	900MHz	*Drosophila* hsp708, affects development, reproduction
Winker et al, 2005 Mutation Res	intermittent 50Hz	human diploid fibroblasts micronuclei, chromosomal damage

Table 2 Biological Thresholds in the ELF Range

Biological System	**Threshold***	**Reference**
Enzyme reaction rates		
Na,K-ATPase	**.2-.3μT**	**Blank & Soo, 1996**
cytochrome oxidase	**.5-.6μT**	**Blank & Soo, 1998**
ornithine decarboxylase	**~2μT**	**Mullins et al, 1999**
Oxidation-reduction rate		
Belousov-Zhabotinsky	**<.5μT**	**Blank & Soo, 2001b**
Biosynthesis of stress proteins		
HL60, Sciara, yeast,	**<.8μT**	**Goodman et al, 1994**
breast (HTB124, MCF7)	**<.8μT**	**Lin et al, 1998**
chick embryo (anoxia)	**~2μT**	**DiCarlo et al, 2000**
Disease related **block melatonin inhibition of breast carcinoma**	**.2<1.2μT**	**Liburdy et al, 1993**
leukemia epidemiology	**.3-.4μT**	**Ahlbom et al, 2000** **Greenland et al, 2000**

*The estimated values are for departures from the baseline, although Mullins et al (1999) and DiCarlo et al (2000) generally give inflection points in the dose-response curves. The leukemia epidemiology values are not experimental and are listed for comparison.

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 7

The Cellular Stress Response: EMF-DNA Interaction

2012 Supplement

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ABSTRACT

The research on stress proteins stimulated by EMF was reviewed by the author in the BioInitiative Report (2007) as well as in the special issue of Pathophysiology (2009) devoted to EMF. This review emphasizes the more recent research on the mechanism of interaction of EMF with DNA. It appears that the DNA molecule is particularly vulnerable to damage by EMF because of the coiled-coil configuration of the compacted molecule in the nucleus. The unusual structure endows it with the self similarity of a fractal antenna and the resulting sensitivity to a wide range of frequencies. The greater reactivity of DNA with EMF, along with a vulnerability to damage, underscores the urgent need to revise EMF exposure standards in order to protect the public. Recent studies have also exploited the properties of stress proteins to devise therapies for limiting oxidative damage and reducing loss of muscle strength associated with aging.

I. INTRODUCTION

The cellular stress response is a protective reaction of individual cells to potentially harmful stimuli in the environment. It is characterized by the synthesis of a class of proteins referred to as stress proteins. The cellular stress response differs from the more familiar responses of entire organisms to stresses that lead to secretion of cortisol and adrenalin and that result in the activation of various systems throughout the body. The cellular stress response, as the name indicates, is a specific response of individual cells, and stress proteins are the chemical agents that also serve as markers.

The cellular stress response was first described as a reaction to elevated temperature (Ritossa, 1962), which accounts for the proteins initially being called heat shock proteins. Several physical and chemical environmental influences have since been found to evoke the response, and in 1994, Goodman and Blank (1994) were the first to show that the response was stimulated by EMF. In fact, the cells were far more sensitive to EMF than to thermal stimuli, the threshold energy of the EMF stimulus being more than one billion times weaker than an effective thermal stimulus (Blank , Goodman, 1994).

The ‘heat shock’ response, i.e., hsp synthesis, is activated by a variety of potentially harmful stresses, including physical stimuli like pH and osmotic pressure changes, as well as chemicals such as ethanol and toxic metal ions like Cd^{2+}. The ability of EMF in the power frequency (extremely low frequency, ELF) range (Goodman, Blank, 1998) to evoke this response was followed by reports of similar effects due to radio frequency (RF) fields (de Pomerai et al. 2003) and amplitude modulated RF fields (Czyz et al, 2004).

The finding that EMF evoked the cellular stress response had obvious and important biological implications:

- Because the cellular stress response is a reaction to potentially harmful stimuli in the environment, the cells were asserting that *EMF is potentially harmful* to cells.
- Because EMF stimulated protein synthesis, it meant that *EMF causes the two strands of DNA to come apart* for the protein code to be read and for synthesis to proceed.
- Since *EMF can interact with DNA*, it can cause *errors during replication,* as well as during protein synthesis, and higher energy EMF could be expected to cause *DNA strand breaks*, as has been observed (Lai and Singh, 1995).
- The incremental increase of DNA strand breaks with increases in field strength indicates a *dose-response*, evidence in support of EMF as the responsible agent.

II. CELLULAR STRESS PROTEINS ARE A NEW CLASS OF PROTEINS

Proteins are important components of cells and make up about 50% of the dry weight of most cells. The many different proteins are classified according to their functions, and stress proteins are now recognized as a new class of proteins with functions related to cell protection. Stress proteins join such well-known categories as contractile proteins (e.g. actin, myosin), catalytic proteins or enzymes (e.g. pepsin, amylase), transport proteins (e.g. ATPases for ions across membranes, hemoglobins for blood gases, cytochromes for electrons), etc. Stress proteins were originally described as being synthesized in response to external stimuli and that is currently the area of greatest interest. However, they are also present constitutively.

Cellular stress proteins are synthesized when cells come in contact with stimuli that cause damage to macromolecules (Kultz, 2005), and the stress proteins aid in the repair and transport of these molecules. Because the first stimulus identified was an increase in temperature, the proteins were called 'heat shock' proteins and designated using the original terminology that starts with 'hsp' (for 'heat shock' protein) and a number equal to the molecular weight in kilodaltons.

The transition from heat shock protein to stress protein should alert (perhaps even alarm) the government agencies responsible for setting EMF safety standards. The thermal stimuli that evoked synthesis of protective proteins were believed to be dangerous for cells, but now we see that non-thermal EMF stimuli cause the same protective reactions in cells. The heat shock response and the EMF stress response both relate to the threshold for biological damage, and we should realize that EMF damage is caused by non-thermal stimuli. Compared to the energy needed to stimulate heat shock, EMF requires but a small fraction of the thermal energy needed to produce the same response (Blank et al., 1992).

The government agencies that assess safety of EMF exposure assume that danger is associated with an increase in temperature, i.e., a thermal criterion. It is clear from the responses of cells that the safety of EMF exposure, as indicated by the synthesis of protective stress proteins, is unrelated to the temperature increase. The cells are very sensitive to EMF, and the protective biological response to EMF occurs long before there is a significant change in temperature. It should be obvious that EMF safety standards are based on false assumptions and must be revised to reflect the scientific evidence. Non-thermal EMF stimuli are potentially harmful.

III. PROTEIN SYNTHESIS

The stress response, like all protein synthesis, indicates that all of the different physical and chemical stimuli that can initiate this response cause the two strands of DNA to come apart for the amino acid code for protein synthesis code to be read. Therefore, the observed stress protein synthesis is evidence that EMF has interacted with the DNA to start this process. The research showing that EMF in both the ELF and RF frequency ranges can also cause DNA strand breaks (Lai, Singh, 1995; 1996; Reflex Report 1994), suggests that the two phenomena are due to the same interaction mechanism, and that there is greater molecular damage with greater EMF energy.

Many research papers and some reviews have been published since the cellular stress response was reported to be stimulated by EMF. In addition to earlier reviews on EMF stimulation of the cellular stress response in the ELF (Goodman, Blank, 1998) and RF (Cotgreave, 2005) ranges, the subject was reviewed in Pathophysiology (Blank, 2009). Also, Calderwood (2007) has edited the volume on cell stress proteins in volume 7 of the series Protein Reviews. A recent (ICEMS, 2010) review on EMF and Bio-Effects includes many papers focused on a variety of possible EMF interaction mechanisms, but does not review the stress response, the stimulation of DNA or biosynthesis.

Section 7 of the Bioinitiative Report summarized both ELF and RF studies, mainly at frequencies 50 Hz, 60 Hz, 900MHz and 1.8 GHz. The citations in that review were not exhaustive, but the different frequencies and many different cells indicated the diversity of results on stimulation of DNA and stress protein synthesis. The many different types of cells that respond to EMF, both *in vivo* and *in vitro*, include epithelial, endothelial and epidermal cells, cardiac muscle cells, fibroblasts, yeast, *E. coli*, developing chick eggs, and dipteran cells.

It is clear that the stress response does not occur in reaction to EMF in all types of cells, and that tissue cultured cells (as opposed to natural cells) are less likely to show an effect of EMF, probably because immortalized cells have been changed significantly to enable them to live indefinitely in unnatural laboratory conditions. Even the same cell line from two different suppliers can respond differently. Jin et al. (1997) showed that HL60 cells from one supplier reacted to EMF while identically labeled cells from another supplier did not respond. Some cancer cells (e.g., MCF7 breast cancer cells) have responded to EMF (Liburdy et al., 1993; Lin et al., 1998), and Czyz et al. (2004) found that p53-deficient embryonic stem cells showed an increased EMF response, but the wild type did not. Ivanscits et al., 2005) found no genotoxic effects (i.e., DNA damage) in lymphocytes, monocytes and skeletal muscle cells, but did find effects with fibroblasts, melanocytes and rat granulosa cells. Lantow et al. (2006) and Simko et al. (2006) found that blood elements, such as lymphocytes and monocytes did not respond. Obviously, the cellular stress response is widespread but not universal.

IV. MECHANISM OF PROTEIN SYNTHESIS BY EMF

The stress response has provided an opportunity to investigate EMF interaction with DNA, and in particular, how this results in stimulating DNA to start the synthesis of proteins. Because the DNA sequence is known for hsp70, it was possible to study the effects of changes in the DNA sequence on protein synthesis. As a result of these experiments, it was possible to identify two distinct regions in the promoter region of the HSP 70 gene - an EMF sensitive region that was not sensitive to increased temperature, as well as a region sensitive only to temperature. The EMF sensitive domain contains number of nCTCTn myc-binding sites relative to the transcription initiation site and upstream of the temperature sensitive binding sites (Lin et al. 1999; 2001). These electromagnetic response elements (EMREs) are also found on the c-*myc* promoter which also reacts to EMF.

The EMF sensitivity of the DNA sequences, nCTCTn, was demonstrated by transfecting these sequences into CAT and Luciferase reporter genes and stimulating those genes (with EMF) to synthesize CAT and luciferase, respectively (Lin et al., 1999; 2001). Thus, the HSP70 promoter contains different DNA regions that are specifically sensitive to thermal and non-thermal stressors. This biological mechanism is obviously based on direct interaction with specific segments of DNA, and there is reason to believe that EMF can interact similarly with other segments of DNA. In our experiments, induction of increased levels of hsp70 by EMF is rapid and occurs at extremely low levels of energy input, 14 orders of magnitude lower than with a thermal stimulus (Blank et al. 1994).

V. EMF INTERACTION WITH SIGNALING PATHWAYS

EMF penetrate cells unattenuated and so can interact directly with the DNA in the cell nucleus, as well as with other cell constituents. The above-cited experiments demonstrating the ability of electromagnetic response elements (EMREs) to interact with EMF, after being transferred to another DNA chain, is further support for direct EMF-DNA interaction as the most likely mechanism for EMF initiation of the cellular stress response.

In contrast to EMF, most biological agents are impeded by membranes and require special mechanisms to gain access to the cell interior. Friedman et al, (2007) have demonstrated that, in those situations, the initial step in transmitting extracellular information from the plasma membrane to the nucleus of the cell occurs when NADH oxidase rapidly generates reactive oxygen species (ROS). These ROS stimulate matrix metalloproteinases that allow them to cleave and release heparin binding epidermal growth factor. This secreted factor activates the epidermal growth receptor, which in turn activates the extracellular signal regulated kinase 1\2 (ERK) cascade. The ERK cascade is one of the four mitogen-activated protein kinase (MAPK) signaling cascades that regulate transcriptional activity in response to extracellular stimuli.

Stress protein synthesis can occur by direct interaction of EMF with DNA, as well as by membrane mediated stimulation via chemical signaling. While both mechanisms are possible, it is of interest to note that the body responds directly to physical inputs when there is a need for a rapid response. The body cannot rely upon slowly responding pathways for the synthesis of a relatively large amount of urgently needed protein molecules. The signal pathways function primarily as a mechanism for maintaining homeostasis by minimizing change and responding slowly to stimuli.

VI. INSIGHTS FROM MUSCLE PROTEIN SYNTHESIS

EMF stimulated protein synthesis may appear to be an unnatural mechanism, but it is essentially the same as the natural process in striated muscle. The only difference is that the electrons in DNA are driven by EMF, while in striated muscle, they are driven by the changes in electric (membrane) potential that cause contraction. Striated muscle is a tissue that requires steady protein synthesis to ensure proper function. Protein synthesis is initiated by the same electric currents that stimulate the muscle contractions. Body builders know that one must stimulate muscle contraction in order to increase muscle mass, and biologists have shown that the electric currents that flow across the muscle membranes during contraction pass through the DNA in the muscle nuclei and stimulate protein synthesis.

Muscle nuclei are not spread evenly throughout a muscle fiber, but are located near the muscle membranes that carry the currents. This means that the DNA in the nuclei can be stimulated every time the muscle is stimulated. The estimated magnitude of electric field along the muscle nuclei, ~10V/m, provides a large safety margin in muscle, since fields as low as 3mV/m were found to stimulate biosynthesis in HL60 cells (Blank et al, 1992).

Studies showing effects of EMF on electron transfer reactions in solution suggest that ionic (electric) currents affect electron movements within DNA in much the same way (Blank, 1995). Both electric and EMF (AC magnetic fields) stimulate protein synthesis in HL60 cells and have similar effects on electron transfer in the Na,K-ATPase (Blank and Soo, 2001a; 2001b). This suggests that interaction with DNA, of both electric fields and EMF, initiate stress protein synthesis by a similar mechanism.

Studies on muscle protein synthesis also suggest the possibility of a frequency code that controls the particular segment of DNA that is activated. Studies have shown that different proteins can be synthesized by changing the frequency of the action potentials that stimulate the process. These experiments were possible because ‘fast’ and ‘slow’ muscles contract at different rates because they are composed of different proteins. For this reason it was possible to stimulate muscles at different rates and to study changes in the proteins as a result of changing the frequency of the action potentials (Pette, Vrbova, 1992). The review by Blank (1995) includes many additional experiments that show the importance of the frequency in controlling the segment of the muscle DNA that is affected by the current and translated into protein.

Studies of effects of EMF on well characterized electron transfer reactions, involving cytochrome oxidase, ATP hydrolysis by Na,K-ATPase, and the Belousov–Zhabotinski (BZ) redox reaction, have shown that:

- EMF can accelerate electron transfer rates
- EMF acts as a force that competes with the chemical forces driving a reaction. This means that the effect of EMF varies inversely with the intrinsic reaction rate, and that EMF effects are only seen when intrinsic rates are low. (*N.B. EMF has a greater effect when the system is in a rundown state.*)
- Experimentally determined thresholds are low (~0.5μT).
- Effects vary with frequency, with different optima for the reactions studied: The two enzymes showed broad frequency optima close to the reaction turnover numbers for Na,K-ATPase (60 Hz) and cytochrome oxidase (800 Hz), suggesting that EMF interacted optimally when in synchrony with the molecular kinetics. EMF interactions with DNA in both ELF and RF ranges and do not appear to involve electron transfer reactions with well-defined kinetics.

The effects of EMF on electron transfer reactions were studied in the ELF frequency range, and one would expect differences in the RF range. However, the situation is more complicated. The effects of EMF on electrons in chemical reactions were detected in the Na,K-ATPase when electric or magnetic fields, each accelerated the reaction only when the enzyme was relatively inactive, i.e., the chemical driving forces were weak. These experiments enabled an estimate of the electron velocity as approximately 10^3 m/s (Blank and Soo, 2001a; 2001b), a velocity similar to that of electrons in DNA. An electron moving at a velocity of 10^3 m/s crosses the enzyme ($\sim 10^{-8}$ m) before the ELF field has had a chance to change. This means that a low frequency effect on fast moving electrons in DNA or in enzymes should be viewed as effectively due to a repeated DC pulse. In the RF range, the pulse train is longer.

VII. DNA IS A FRACTAL ANTENNA

Human DNA is about 2 m long, and the molecule is greatly compacted so that it fits into the nuclei of cells that are microns in diameter.

DNA has a unique double helical structure where two strands of DNA are bound together by hydrogen bonds between pairs of nucleotide bases (one on each strand) and they form a long twisted ribbon with delocalized π electrons that form continuous planar clouds on both surfaces of the ribbon. The result is a structure with two continuous paths that can conduct an electron current along the DNA.

Many studies, initially from the laboratory of Barton at Cal Tech (Hall et al, 1996), have shown that DNA does indeed conduct electrons. As would be expected, the rate of conduction can be influenced by the detailed structure of DNA. Changes, such as hairpin turns and mismatched bases, can lead to the disruption of the ordered double helical structure and anomalies in the rate of electron flow (Arkin et al, 1996; Hall et al, 1997; Lewis et al, 1997; Kelley et al, 1999; Giese, 2002). Electron flow can lead to local charging as well as oxidative damage.

Variations in the rate of electron flow can lead to the accumulation of charge at bottlenecks. The temporary buildup of charge at a site results in strong repulsive forces that can cause a disruption of H-bonds. A net charge can even disrupt the structure of a complex molecule, such as occurs when the four protein chains of hemoglobin disaggregate in response to a gradual buildup of charge in the hemoglobin tetramer (Blank, 1984; Blank and Soo, 1998). For similar reasons, one would expect disaggregating forces at the DNA site where charge builds up. This would be expected to occur more easily in a compact structure such as DNA in the nucleus.

The tightly coiled DNA in the nucleus uses fractal patterns in order to occupy space efficiently. A fractal is a shape that displays *self-similarity*, where each part of the shape resembles the entire shape. Thus, the double helix is wound into a coil and that coil is wound into a larger coil, and so on. DNA in a cell nucleus is a coiled-coil many times over.

Since the DNA molecule in the nucleus conducts electricity and is organized in a self-similar pattern, it has the two key characteristics of *fractal antennas* when interacting with EMF (Blank, Goodman 2011). Fractal design is desirable for an antenna because it minimizes the overall size, while reacting to a wide range of electromagnetic frequencies. However, these characteristics are not desirable in DNA, because of the many frequencies in the environment that can and do react with DNA. The almost continuous cloud of delocalized electrons along both faces of the 'ribbon' formed by the base pairs provides a conducting path for responding to EMF and makes it more vulnerable to damage. The chemical changes that result from electron transfer reactions, are associated with molecular damage in DNA.

VIII. DNA DAMAGE AND CANCER

Stress proteins are essential for cell protection. They help defend cells against damaging forces like increases in temperature and reductions in oxygen supply that could be life-threatening. Similarly, the body generates stress proteins to strengthen cellular resistance to the effects of EM radiation. However, stress protein synthesis is really only an emergency measure that is designed to be effective in the short term. The response to repeated stimuli diminishes with repeated exposure and this could be dangerous.

Thermotolerance, the ability to tolerate higher temperatures as a result of repeated exposures to high temperature, was originally demonstrated at the molecular level in connection with heat shock. Repeated exposure to increased temperature resulted in a decreased heat shock response. A similar mechanism applies when the cellular stress response is stimulated by EMF, since repeated EMF stimuli result in lower production of stress proteins. This could very well be a mechanism by which repeated exposure to EMF can result in less protection and more damage to molecules like DNA. The lower protection predisposes exposed individuals to an increased risk of mutation and initiation of cancer.

DiCarlo and Litovitz (2008) at Catholic University in Washington, D.C. demonstrated the development of EMF tolerance in an experiment performed on chicken embryos. In those eggs exposed to ELF-radiation of 8 µT for 30 or 60 minutes at a time, twice a day for four days, production of hsp70 in response to oxygen deprivation declined. The same response was noted in those eggs exposed to RF radiation of 3.5 $\mu W/cm^2$ for 30 or 60 minutes, once a day, for four days. The researchers noted that these eggs produced 27% less hsp70 following these exposures, and had correspondingly reduced ability to fend off cell damage (reduced *cytoprotection*). Similar experiments have been carried out with short, repeated exposures (in contrast to extended exposures). There too, the rate of stress protein synthesis is reduced with each repetition. The reduction in stress protein synthesis as a result of continuous exposure to EMF would predispose an individual to the accumulation of DNA damage and the development of cancer.

Cancers are believed to be the long term result of the errors in DNA that occur during the normal functioning of cells. Living cells are continuously growing (making protein) and dividing (making DNA), and errors in synthesis occur. The error rate is a very small but finite, so the vast majority of errors is repaired, but not all. When the error rate is too high, the cell activates apoptosis and destroys itself . However, the small number of errors that is retained accumulates over time as mutations, some of which can affect function. It is particularly bad when mutation inactivates a tumor suppressor gene or a

DNA repair gene and enables creation of an oncogene, since this accelerates the development of a cancer.

Although damage can occur during protein synthesis and cell division, as well as upon exposure to oxidizing chemicals, the probability of developing cancer is increased as a result of damage to DNA structure caused by exposure to EMF (Verschaeve, 2008). EMF induced oxidative damage to DNA has even been reported on exposure to high ELF fields (Yokus et al, 2008).

IX. STRESS RESPONSE: BIOLOGICAL GUIDE TO SAFETY

The cellular stress response is the way the body tells us that it has come in contact with a potentially harmful stimulus. Since cells react to relatively low levels of EMF, both ELF and RF, one would think that the low biological thresholds for a protective reaction to harmful stimuli would provide critical guidance for the authorities seeking to establish meaningful safety standards. By ignoring the information from the cellular stress response, the authorities appear to be saying that they are better judges of what is harmful to cells than the cells themselves.

Research on the cellular stress response has drawn attention to the inadequacy of EMF safety standards. The synthesis of stress proteins at EMF levels that are currently considered safe indicates that ambient exposure levels can influence the molecular processes involved in protein synthesis needed to provide new molecules and replace damaged molecules. The ability of EMF to interfere with normal function and damage the protein and DNA molecules that are being synthesized is definitely a reason to consider this effect for guidance regarding its health implications. The system of safety standards is not at all protective because processes stimulated at non-thermal levels have been overlooked. The standards must be revised.

The authorities have been misguided in assuming that only thermal stimuli could affect chemical bonds and that non-thermal stimuli cannot cause chemical changes. Non-thermal biological mechanisms activated by EMF have been known for some time, and some experiments have even been aimed specifically at demonstrating unusual changes in biological systems due to non-thermal EMF stimuli. Bohr and Bohr (2000) showed that both a reaction and its reverse, the denaturation and renaturation of β-lactoglobulin, are accelerated by microwave EMF, and de Pomerai et al (2003) showed that microwave radiation causes protein aggregation in the absence of bulk heating. A clear separation of thermal and non-thermal mechanisms in biology was shown by Mashevich et al (2002) in experiments where chromosomal damage in lymphocytes that had been observed under RF was not seen when the cells were exposed to elevated temperatures. The neglect of non-thermal mechanisms by regulators is based on their ignorance of reactions in biological systems. By greatly underestimating the risk of EMF exposure, they continue to endanger the public.

The cellular stress response is activated by a mechanism that involves interaction of EMF with the DNA molecule. This reaction of DNA, and/or the stress proteins that are synthesized, could be used to develop new EMF safety standards (Blank and Goodman, 2012). A biologically-based measure of EMF radiation could replace the misguided energy-based "specific absorption rate" (SAR). (It should be noted that SAR is the safety standard in the radiofrequency (RF) range, but it fails as a standard for predicting cancer risk in the ELF range.) A standard based on stress proteins would have several advantages compared to SAR:

- it is based on a protective cellular mechanism that is stimulated by a variety of potentially harmful environmental agents
- it is stimulated by a wide range of frequencies in the EM spectrum so there would be no need for different standards in different frequency ranges.

Cancers are believed to arise from mutations in DNA, and changes in DNA induced by interaction with EMF could be a better measure of the biologically effective dose. It may be possible to measure the changes by transcriptional alterations and/or translational changes in specific proteins. A biologically-based standard related to stimulation of DNA could apply over a much wider range of the electromagnetic spectrum and include ionizing radiation.

X. STRESS RESPONSE: GUIDE TO NEW THERAPIES

Since activation of the cellular stress response by EMF was shown to be a protective mechanism, it was only a matter of time before the response would be studied as a potential therapeutic agent. Thermal activation of the stress response has already been shown to be effective in cardiac bypass surgery (Currie et al., 1993; Udelsman et al., 1993; Nitta et al., 1994). Stress protein activation can apparently minimize the oxidative damage of ischemia (low oxygen level in a tissue) reperfusion that occurs when the blood supply is reconnected to the heart after surgery. However, the temperature control required for thermal activation is cumbersome and the technique is not easily applied compared to EMF. A study of non-invasive EMF induction of hsp70, prior to cardiac bypass surgery, has shown that myocardial function can be preserved, and at the same time decrease ischemic injury (George et al, 2008).

EMF activation of stress protein synthesis has a clear advantage over thermal activation. The biological response is not related to the EMF energy, so protective biological responses should occur far below thermal levels. 60 Hz fields were shown to induce elevated levels of hsp70 protein in the absence of elevated temperature (Goodman et al., 1994; Goodman and Blank, 1998; Han et al., 1998; Lin et al., 1998, 1999, 2001; Carmody et al., 2000) in cells including cultured rodent cardiomyocytes (Goodman and Blank, 2002). Also, Di Carlo et al. (1999) and Shallom et al. (2002) confirmed that cardiomyocytes were protected from anoxic damage in EMF exposed chick embryos.

Another potential therapeutic application has come from a study of the stress protein hsp10 in relation to striated muscle function. Kayani et al (2010) at the University of Liverpool found that this stress protein can prevent the age-related deterioration of muscle strength in skeletal muscle of transgenic mice. Hsp10 is often linked with hsp60 in supporting mitochondrial function. In cardiac myocytes this combination protects mitochondrial function as well as preventing cell deaths induced by ischemia-reperfusion.

These results suggest that mitochondrial hsp10 and hsp60 in combination or individually play an important role in maintaining mitochondrial integrity and ability to generate ATP, which are crucial for survival of cardiac myocytes during ischemia/reperfusion.

Research on therapeutic effects using stress proteins is obviously just beginning and we can expect other applications where EMF is used to generate this group of therapeutic agents essentially instantaneously and in situ.

XI. THE ENVIRONMENTAL EMF ISSUE AND CONCLUSIONS

Research has shown that the EMF-activated cellular stress response:

- is an effective protective mechanism for cells exposed to a wide range of EMF frequencies
- thresholds are very low (safety standards must be reduced to limit biological responses)
- mechanism involves direct interaction of EMF with the DNA molecule (claims that there are no known mechanisms of interaction are patently false)
- the coiled-coil structure of DNA in the nucleus makes the molecule react like a fractal antenna to a wide range of frequencies (there is a need for stricter EMF safety standards)
- biologically-based EMF safety standards could be developed from the research on the stress response.

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SECTION 8

Evidence For Effects On The Immune System

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Appendix 8-A Some legal aspects of the functional impairment electrohypersensitivity in Sweden

I. Basic concepts and components of the immune system

The human immune system is part of a general defense barrier towards our surrounding environment. We live in a biological system, the world, dominated by various microorganisms, including microbes and viruses, many of which can cause harm. The immune system serves as the primary line of defense against invasion by such microbes. As we are, practically speaking, built as a tube, the outer surface - the skin - and the innermost surface - the gastrointestinal tract - are the major borders between us and the rest of the universe. These borders must be guarded and protected since any damage to them could be fatal.

The skin and the mucous membranes are part of the innate or non-adaptive immune system. However, if these barriers are broken (e.g. after cutting a finger), then microbes, including potential pathogens (i.e. harmful microbes) can enter the body and then begin to multiply rapidly in the warm, moist, nutrient-rich environment. The cut may not be as physical, brutal and abrupt as a knife cut, it could also very well be an internal leakage, such as the one found after microwave exposure of the fragile blood-brain-barrier (cf. Persson et al, 1997). Such a leakage could indeed be fatal, causing nerve cell damage and consecutive cellular death (cf. Salford et al, 2003).

One of the first cell types to be encountered by a foreign organism after a cut in the skin is the phagocytic white blood cells which will congregate within minutes and begin to attack the invading foreign microbes. Following this, the next cell type to be found in the area of such a local infection will be the so-called neutrophils. They are also phagocytic and use pattern-regonizing surface receptor molecules to detect structures commonly found on the surface of bacteria. As a result, these bacteria - as well as other forms of particulate materia - will be ingested and degraded by the neutrophils. Various other protein components of serum, including the complement components may bind to the invader organisms and facilitate their phagocytosis, thereby further limiting the source of infection/disease. Other small molecules, the interferons, mediate an early response to viral infection by the innate system.

The innate immune system is often sufficient to destroy invading microbes. If it fails to clear an infection, it will rapidly activate the adaptive or acquired immune response, which - as a consequence - takes over.The molecular messenger connection between the innate and the adaptive systems are molecules known as cytokines (actually, the interferons are part of this molecular family).

The first cells in this cellular orchestra to be activated are the T and B lymphocytes. These cells are normally at rest and are only recruited at need, i.e. when encountering a foreign (=non-self) entity referred to as an antigen. The T and B lymphocytes, together with a wide spectrum of other cell types, have antigen receptors or antigen-recognizing molecules on their surface. Among them you find the classical antibodies (=B cell antigen receptors), T cell antigen receptors as well as the specific protein products of special genetic regions (=the major histocompatibility complexes). The genes of humans are referred to as human leukocyte antigen (HLA) genes and their protein products as HLA molecules. The antibodies - apart from being B cell surface receptors - are also found as soluble antigen-recognizing molecules in the blood (immunoglobulins). The adaptive immune response is very highly effective but rather slow; it can take 7-10 days to mobilize completely. It has a very effective pathogen (non-self) recognition mechanism, a molecular memory and can improve it's production of pathogen-recognition molecules during the response.

A particularly interesting set of cells are the various dendritic cells of the skin. In the outermost portion, the epidermis, you find both dendritic melanocytes, the cells responsible for the pigment-production, as well as the Langerhans cells with their antigen-presenting capacity. In the deeper layer, the dermis, you find corresponding cells, as well as the basophilic mast cells, often showing a distinct dendritic appearance using proper markers such as chymase, tryptase or histamine. All these cells are the classical reactors to external radiation, such as radioactivity, X-rays and UV light. For that reason, our demonstration (Johansson et al, 1994) of a high-to-very high number of somatostatin-immunoreactive dendritic cells in the skin of persons with the functional impairment electrohypersensitivity is of the greatest importance. Also, the alterations found in the mast cell population of normal healthy volunteers exposed in front of ordinary house-hold TVs and computer screens (Johansson et al, 2001) are intriguing, as are the significantly increased number of serotonin-positive mast cells in the skin ($p<0.05$) and neuropeptide tyrosine (NPY)-containing nerve fibers in the thyroid ($p<0.01$) of rats exposed to extremely low-frequency electromagnetic fields (ELF-EMF) compared to controls, indicating a direct EMF effect on skin and thyroid vasculature (Rajkovic et al, 2005a,b, 2006; for further details and refs., see below). In the gastrointestinal tract, you will find corrsponding types of cells guardening our interior lining towards the universe.

In essence, the immune system is a very complex one, built up of a large number of cell types (B and T lymphocytes, macrophages, natural killer cells, mast cells, Langerhans cells, etc.) with certain basic defense strategies. It has evolved during an enormously long time-span and is constructed to deal with it's known enemies, including bacteria. Among the known enemies are, of course, not modern electromagnetic fields, such as power-frequent electric and magnetic fields, radiowaves, TV signals, mobile phone or Wi-Fi microwaves, radar signals, X-rays or radioactivity. They have been introduced during the last 100 years, in many cases during the very last decades. They are an entirely new form of exposure and could pose to be a biological ”terrorist army” against which there are no working defence walls. They do penetrate the body from outside and in. Some of them have already been proven to be of fatal nature, and today no-one would consider having a radioactive wrist watch with glowing digits (as you could in the 1950s), having your children’s shoes fitted in a strong X-ray machine (as you could in the 1940s), keeping radium in open trays on your desk (as scientists could in the 1930s), or X-raying each other at your garden party (as physicians did in the 1920s). That was, of course, just plain madness. However, the persons doing so and selling these gadgets were not misinformed or less intelligent, not at all. The knowledge at the time was just lacking as was a competent risk analysis behaviour coupled to a parallel analysis of true public need.

II. Hypersensitivity reactions

The immune system can react in an excessive manner and it can cause damage to the local tissue as well as generally to the entire body. Such events are called hypersensitivity reactions and they occur in response to three different types of antigens: a) infectious agents, b) environmental disturbances, and c) self-antigens. The second one is related to the impact of the new electromagnetic fields of today's modern world. Hypersensitivity can occur in response to innocuous environmental antigens - one example of this is allergy. For example, in hay fever, grass pollens themselves are incapable of causing damage; it is the immune response to the pollen that causes harm.

II A. Hypersensitivity to environmental substances

For environmental substances to trigger hypersensitivity reactions, they must be fairly small in order to gain access to the immune system. Dust triggers off a range of responses because they are able to enter the lower extremities of the respiratory tract, an area that is rich in adaptive immune-response cells. These dusts can mimic parasites and may stimulate an antibody response. If the dominant antibody is IgE, they may subsequently trigger immediate hypersensitivity, which is manifest as allergies such as asthma or rhinitis, If the dust stimulates IgG antibodies it may trigger off a different kind of hypersensitivity, e.g. farmer's lung.

Smaller molecules sometimes diffuse into the skin and these may act as haptens, triggering a delayed hypersensitivity reaction. This is the basis of contact dermatitis caused by nickel.

Drugs administered orally, by injection or onto the surface of the body can elicit hypersensitivity reactions mediated by IgE or IgG antibodies or by T cells. Immunologically mediated hypersensitivity reactions to drugs are very common and even very tiny doses of drugs can trigger life-threatening reactions. These are well classified as idiosyncratic adverse drug reactions.

In this respect, of course electromagnetic fields could be said to fulfil the most important demands: they can penetrate the entire body and if they are small.

II B. Hypersensitivity to self antigens

Some degree of immune response to self antigens is normal and is present in most people. When these become exaggerated or when tolerance to further antigens breaks down, hypersensitivity reactions can occur and manifest themselves as an autoimmune disease, many of which that are truly serious and may even end fatally.

II C. Types of hypersensitivity reactions

The hypersensitivity classification system was first described by Coombs and Gell. The system classifies the different types of hypersensitivity reaction by the types of immune responses involved. Each type of hypersensitivity reaction produces characteristic clinical diseases whether the trigger is an enviromental, infectious or self-antigen. For example, in type III hypersensitivity the clinical result is similar whether the antigen is streptococcus, a drug or an autoantigen such as DNA.

Hypersensitivity reactions are reliant on the adaptive immune system. Prior exposure to antigen is required to prime the adaptive immune response to produce IgE (type I), IgG (type II and III) or T cells (type IV). Because prior exposure is required, hypersensitivity reactions do not take place when an individual is first exposed to antigen. In each type of hypersensitivity reaction the damage is caused by different adaptive and innate systems, each of which with their respective role in clearing infections.

Type I

Type I hypersensitivity is mediated through the degranulation of mast cells and eosinophils. The effects are felt within minutes of exposure and this type of hypersensitivity is sometimes referred to as immediate hypersensitivity and is also known as allergy. Among such reactions are hay fever and the classical skin prick test that can be used to reveal such reaction patterns. –The mast cell is a common denominator in the functional impairment electrohypersensitivity (earlier referred to as ”electrical allergy”).

Type II

Type II hypersensitivity is caused by IgG reacting with antigen present on the surface of cells. The bound immunoglobulin then interacts with complement or with Fc receptors on macrophages. These innate mechanisms then damage the target cells using processes that may take several hours, as in the case of drug-induced hemolysis.

Type III

Immunoglobulin is also responsible for the type III hypersensitivity. In this case, immune complexes of antigen and antibody form and either cause damage at the site of production or circulate and cause damage elsewhere. Immune complexes take some time to form and to initiate tissue damage. Among the cells types involved are neutrophils. Post-streptococcal glomerulonephritis is a good example of immune complex disease.

Type IV

The slowest form of hypersensitivity is that mediated by T cells (type IV hypersensitivity). This can take 2-3 days to develop and is referred to as delayed hypersensitivity. Macrophages are frequently involved. A well-known example of such delayed reactions is contact dermatitis.

III. The old and new electromagnetic environment

"Electromagnetic radiation" covers a broad range of frequencies (over 20 orders of magnitude), from low frequencies in electricity supplies, radiowaves and microwaves, infrared and visible light, to x-rays and cosmic rays.

III A. Definitions and sources

Electric fields are created by differences in voltage: the higher the voltage, the stronger will be the resultant field. Magnetic fields are created when electric current flows: the greater the current, the stronger the magnetic field. An electric field will exist even when there is no current flowing. If current does flow, the strength of the magnetic field will vary with power consumption but the electric field strength will be constant.

III B. Natural sources of electromagnetic fields

Electromagnetic fields are present everywhere in our environment but are invisible to the human eye. Electric fields are produced by the local build-up of electric charges in the atmosphere associated with thunderstorms. The earth's magnetic field causes a compass needle to orient in a North-South direction and is used by birds and fish for navigation.

III C. Human-made sources of electromagnetic fields

Besides natural sources the electromagnetic spectrum also includes fields generated by human-made sources: X-rays are employed to diagnose a broken limb after a sport accident. The electricity that comes out of every power socket has associated low frequency electromagnetic fields. And various kinds of higher frequency radiowaves are used to transmit information – whether via TV antennas, radio stations or mobile phone base stations.

III D. What makes the various forms of electromagnetic fields so different?

One of the main characteristics which defines an electromagnetic field (EMF) is its frequency or its corresponding wavelength. Fields of different frequencies interact with the body in different ways. One can imagine electromagnetic waves as series of very regular waves that travel at an enormous speed, the speed of light. The frequency simply describes the number of oscillations or cycles per second, while the term wavelength describes the distance between one wave and the next. Hence wavelength and frequency are inseparably intertwined: the higher the frequency the shorter the wavelength.

III E. A few basic facts

Field strength: An electromagnetic field consist of an electrical part and a magnetic part. The electrical part is produced by a voltage gradient and is measured in volts/metre. The magnetic part is generated by any flow of current and is measured in Tesla. For example, standing under a power line would expose you to an electrical voltage gradient due to the difference between the voltage of the line (set by the power company) and earth. You would also be exposed to a *magnetic* field proportional to the current actually flowing through the line, which depends on consumer demand. Both types of field give biological effects, but the magnetic field may be more damaging since it penetrates living tissue more easily. Magnetic fields as low as around 2 milligauss (mG) or 0.2 microTesla (a millionth of a Tesla) can produce biological effects. For comparison, using a mobile (cell) phone or a PDA exposes you to magnetic pulses that peak at several tens of microTesla (Jokela et al, 2004; Sage et al, 2007), which is well over the minimum needed to give harmful effects. Because mobile phones and other wireless gadgets are held close to the body and are used frequently, these devices are potentially the most dangerous sources of electromagnetic radiation that the average person possesses.

Frequency: The fields must vary with time, e.g. those from alternating currents, if they are to have biological effects. Extremely low frequencies (ELF) represent power-lines and domestic appliances, and here, just now in June 2007, the WHO again has pointed them out as an area for general caution since they are believed to be one of the causes for children's leukemia. Pulsed or amplitude modulated, at a biologically active lower frequency (i.e. when the radio signal strength rises and falls in time with the lower frequency), high-frequencies are the hallmark of mobile phones, WiFi systems, PDAs, etc.

III F. Electromagnetic fields at low frequencies

Electric fields exist whenever a positive or negative electrical charge is present. They exert forces on other charges within the field. The strength of the electric field is measured in volts per metre (V/m). Any electrical wire that is charged will produce an associated electric field.

This field exists even when there is no current flowing. The higher the voltage, the stronger the electric field at a given distance from the wire.
Electric fields are strongest close to a charge or charged conductor, and their strength rapidly diminishes with distance from it. Conductors such as metal shield them very effectively. Other materials, such as building materials and trees, provide some shielding capability. Therefore, the electric fields from power lines outside the house are reduced by walls, buildings, and trees. When power lines are buried in the ground, the electric fields at the surface are hardly detectable.

Plugging a wire into an outlet creates electric fields in the air surrounding the appliance. The higher the voltage the stronger the field produced. Since the voltage can exist even when no current is flowing, the appliance does not have to be turned on for an electric field to exist in the room surrounding it.

Magnetic fields arise from the motion of electric charges. The strength of the magnetic field is measured in amperes per meter (A/m); more commonly in electromagnetic field research, scientists specify a related quantity, the flux density (in microtesla, µT) instead. In contrast to electric fields, a magnetic field is only produced once a device is switched on and current flows. The higher the current, the greater the strength of the magnetic field.

Like electric fields, magnetic fields are strongest close to their origin and rapidly decrease at greater distances from the source. Magnetic fields are not blocked by common materials such as the walls of buildings.

III G. How do static fields differ from time-varying fields*?*

A static field does not vary over time. A direct current (DC) is an electric current flowing in one direction only. In any battery-powered appliance the current flows from the battery to the appliance and then back to the battery. It will create a static magnetic field. The earth's magnetic field is also a static field. So is the magnetic field around a bar magnet which can be visualized by observing the pattern that is formed when iron filings are sprinkled around it.

In contrast, time-varying electromagnetic fields are produced by alternating currents (AC). Alternating currents reverse their direction at regular intervals. In most European countries electricity changes direction with a frequency of 50 cycles per second or 50 Hertz. Equally, the associated electromagnetic field changes its orientation 50 times every second. North American electricity has a frequency of 60 Hertz.

What are the main sources of low, intermediate and high frequency fields? The time-varying electromagnetic fields produced by electrical appliances are an example of extremely low frequency (ELF) fields. ELF fields generally have frequencies up to 300 Hz. Other technologies produce intermediate frequency (IF) fields with frequencies from 300 Hz to 10 MHz and radiofrequency (RF) fields with frequencies of 10 MHz to 300 GHz. The effects of electromagnetic fields on the human body depend not only on their field level but on their frequency and energy. Our electricity power supply and all appliances using electricity are the main sources of ELF fields; computer screens, anti-theft devices and security systems are the main sources of IF fields; and radio, television, radar and cellular telephone antennas, and microwave ovens are the main sources of RF fields. These fields induce currents within the human body, which if sufficient can produce a range of effects such as heating and electrical shock, depending on their amplitude and frequency range. (However, to produce such effects, the fields outside the body would have to be very strong, far stronger than present in normal environments.)

There are four phenomena that emerge from the use of electricity: ground currents; "electromagnetic smog" from communications equipment; magnetic fields from power lines and specialized equipments; and radiofrequencies on power lines or so-called "dirty electricity." They may all be potential environmental toxins and this is an area of research that must be further pursued.

Electromagnetic fields at high frequencies

Mobile telephones, television and radio transmitters and radar produce RF fields. These fields are used to transmit information over long distances and form the basis of telecommunications as well as radio and television broadcasting all over the world. Microwaves are RF fields at high frequencies in the GHz range. In microwaves ovens, we use them to quickly heat food at 2.45 GHz (or 2,450 MHz).

Communications and radar antennae expose those who live or work near these installations to their emissions. The radiation travels through buildings, and can also be conducted along electrical wires or metal plumbing. Wireless communications create levels within buildings that are orders of magnitude higher than natural background levels.

At radio frequencies, electric and magnetic fields are closely interrelated and we typically measure their levels as power densities in watts per square metre (W/m^2).

IV. The immune system and the impairment electrohypersensitivity

An increasing number of studies has clearly shown various biological and medical effects at the cellular level of electromagnetic fields, including power-frequency and radiofrequency/microwave exposures at low-intensity levels. —Such electromagnetic fields are present in everyday life, at the workplace, in ~~your home~~ in homes and at places of leisure. Such bioeffects and health impacts are substantially documented in the scientific literature, and are directly relevant to public health.

Direct effects on the immune system were first reported in relation to people with symptoms of electrohypersensitivity. Subjective and objective skin- and mucosa-related symptoms, such as itch, smarting, pain, heat sensation, redness, papules, pustles, etc., after exposure to visual display terminals (VDTs), mobile phones, DECT telephones, WI-FI equipments, as well as other electromagnetic devices were reported. Frequently, symptoms from internal organ systems, such as the heart and the central nervous system were reported.

A working definition of EHS from Bergqvist et al. (1997) is:

"a phenomenon where individuals experience adverse health effects while using or being in the vicinity of devices emanating electric, magnetic or electromagnetic fields (EMFs)".

Stenberg (2004) distinguishes between two groups: those who experience facial skin symptoms in connection with VDT work (sensory sensations of the facial skin including stinging, itching, burning, erythema, rosacea) while EHS symptoms include these and also fatigue, headache, sleeplessness, dizziness, cardiac and cognitive problems.

Hillert (2004) reports that symptoms of EHS may include facial skin complaints, eye irritation, runny or stuffy nose, impaired sense of smell, hoarse dry throat, coughing, sense of pressure in ear(s), fatigue, headache, heaviness in the head, nausea/dizziness, and difficulties in concentrating.

Cox (2004) reported on a study of electrical hypersensitivity in the United Kingdom. Symptoms reported by mobile phone users included headaches (85%), dizziness (27%), fatigue (24%), nausea (15%), itching (15%), redness (9%), burning 61%), and cognitive problems (42%). For those individuals reporting EHS symptoms in the UK population, the percentage of patients with symptoms from cell phone masts was 18%, DECT cordless phones (36%), landline phones (6%), VDTs (27%), television (12%) and fluorescent lights (18%).

Fox et al (2004) reported that a questionnaire survey of EHS individuals revealed symptoms of nausea, muzziness/disorientation.

Levallois et al. (2002) reported on their study of prevalence of self-perceived hypersensitivity to electromagnetic fields in California. They found that about 3% of the population reports to be electrohypersensitive. About 0.5% of the population has reported the necessity to change jobs or to remain unemployed due to the severity of their electrohypersensitivity symptoms. Underestimation of these percentages is discussed, since the population surveyed was found through contact with either an occupational clinic or a support group, and electrohypersensitive people very frequently cannot due normal outings (go out, travel, meet in buildings with EMF exposures, etc). The study concludes that while there was no clinical confirmation of the reported symptoms of electrohypersensitivity, the perception is of public health importance in California, and perhaps North America. The results were based on a telephone survey among a sample of 2,072 Californians. Being “allergic or very sensitive” to getting near electrical devices was reported by 68 subjects resulting in an adjusted prevalence of 3.2% (95% confidence interval: 2.8, 3.7). Twenty-seven subjects (1.3%) reported sensitivity to electrical devices but no sensitivity to chemicals. Alleging that a doctor had diagnosed “environmental illness or multiple chemical sensitivity” was the strongest predictor of reporting being hypersensitive to EMF in this population (adjusted prevalence odds ratio = 5.8, 95 % confidence interval: 2.6 - 12.8. This study confirms the presence of this self-reported disorder in North America.

A recent German survey suggests that the prevalence of subjects who attribute health complaints to EMF exposures is not negligible. In a sample of 2,500 interviewees, 8% specifically attributed health complaints to exposures from mobile phone base station antennas or the use of mobile or cordless phones [Institut für angewandte Sozialwissenschaft (infas), 2004]. In Sweden, 3.1% of the population claimed to be hypersensitive to EMF. Considerable variation across countries, regions within countries, and surveys in the same regions has been noted before. In 1997, a European expert group reported that electrical hypersensitivity had a higher prevalence in Sweden, Germany, and Denmark than in the United Kingdom, Austria, and France [European group of experts, 1997]. All these data suggest that the true number is still uncertain and the topic merits further research (cf. Schuz et al, 2006).

Roosli et al. (2004a, 2004b) estimates that the proportion of individuals in Switzerland with EHS symptoms is about 5%, where the exposures of concern are cited to be powerlines, handheld phones, television and computer exposures rather than base stations (cell towers). He reported that about half the Swiss population is concerned about health effects from EMF exposures in general.

V. Scientific studies of electrohypersensitivity, as well as effects of electromagnetic fields on humans

Lyskov et al. (2004) reported that EHS individuals exhibited sensitivity to VDTs, fluorescent lights and television, all of which produce flickering light. EHS individuals that were given provocation tests with flickering light exhibited a higher critical flicker frequency (CFF) than normal, and their visual evoked potential (VEP) was significantly higher than in controls. Follow-up studies, individuals with EHS demonstrated increased CFF, increased VEP, increased heart rate, decreased heart rate variability (HRV) and increased electrodermal (EDA) reaction to sound stimuli. These results indicate an imbalance in the autonomic nervous system and a lack of normal circadian rhythms in these EHS individuals. However, it may also just show that they feel ill.

Mueller and Schierz (2004) reported that soundness of sleep and well-being in the morning but not sleep quality were affected by exposure in EHS individuals to overnight EMF exposures. An effect was reported where EHS individuals shifted their position in the bed during sleep to the non-exposed (or probably less exposed) side of the bed.

Vecchio et al (2007) have reported that EMF from mobile phones affects the synchronization of cerebral rhythms. Their findings suggest that prolonged exposure to mobile phone emissions affect cortical activity and the speed of neural synchronization by interhemispherical functional coupling of EEG rhythms. This may be evidence that such exposure can affect the way in which the brain is able to process information, by interfering with the synchronization rhythms between the halves of the brain, and by disregulating the normal alpha wave 2 (about 8-10 Hz) and alpha 3 (10-12 Hz) bands.

Markova et al. (2005) reported that non-thermal microwave exposure from Global System for Mobile Communication (GSM) mobile telephones at lower levels than the ICNIRP safety standards affect 53BP1 and γ-H2AX foci and chromatin conformation in human lymphocytes. They investigated effects of microwave radiation of GSM at different carrier frequencies on human lymphocytes from healthy persons and from persons reporting hypersensitivity to electromagnetic fields (EMFs). They measured the changes in chromatin conformation, which are indicative of stress response and genotoxic effects, by the method of anomalous viscosity time dependence, and analyzed tumor suppressor p53-binding protein 1 (53BP1) and phosphorylated histone H2AX (γ-H2AX), which have been shown to colocalize in distinct foci with DNA double-strand breaks (DSBs), using immunofluorescence confocal laser microscopy. The authors reported that microwave exposure from GSM mobile telephones affect chromatin conformation and 53BP1/γ-H2AX foci similar to heat shock. For the first time, they reported that effects of microwave radiation from mobile telephones on human lymphocytes are dependent on carrier frequency. On average, the same response was observed in lymphocytes from hypersensitive and healthy subjects. These effects occurred at non-thermal microwave exposure levels from mobile telephones. These levels are presently permissible under safety standards of the International Commission for Non-Ionizing Radiation Protection (ICNIRP).

Recent evidence has indicated activation of stress-induced pathways in cultivated cells in response to microwaves (Leszczynski et al, 2002). Their article indicated that mobile telephone microwaves activate a variety of cellular signal transduction pathways, among them the hsp27/p38MAPK stress response pathway (Leszczynski et al, 2002). Whether activation of stress response pathways relates to apoptosis, blood-brain barrier permeability, or increased cancer in humans remains to be investigated. Further work reported gene and protein expression changes in human endothelial cell lines with microwave 900 MHz mobile phone exposure (Leszczynski and Nylund, 2006).

Persons claiming adverse skin reactions after having been exposed to computer screens or mobile phones very well could be reacting in a highly specific way and with a completely correct avoidance reaction, especially if the provocative agent was radiation and/or chemical emissions -- just as would happen if you had been exposed to e.g. sun rays, X-rays, radioactivity or chemical odors. The working hypothesis, thus, early became that they react in a cellularly correct way to the electromagnetic radiation, maybe in concert with chemical emissions such as plastic components, flame retardants, etc., something later focussed upon by professor Denis L. Henshaw and his collaborators at the Bristol University (cf. Fews et al, 1999a,b). This is also covered in great depth by the author Gunni Nordström in her latest book (2004).

Very early immune cell alterations were observed when exposing two EHS individuals to a TV monitor (Johansson et al, 1994). In this people were placed in front of, in front of an ordinary TV set (an open provocation study). Subjects who regarded themselves as suffering from skin problems due to work at video display terminals were tested. Employing immunohistochemistry, in combination with a wide range of antisera directed towards cellular and neurochemical markers, we observed and reported a high-to-very high number of somatostatin-immunoreactive dendritic cells as well as histamine-positive mast cells in skin biopsies from the anterior neck taken before the start of the provocation. At the end of the provocation the high number of mast cells was unchanged, however, all the somatostatin-positive cells had seemingly disappeared. The reason for this latter finding may be discussed in terms of loss of immunoreactivity, increase of breakdown, etc. The high number of mast cells present may explain the clinical symptoms of itch, pain, edema and erythema.

In facial skin samples of electrohypersensitive persons, the most common finding is a profound increase of mast cells as monitored by various mast cell markers, such as histamine, chymase and tryptase (Johansson and Liu, 1995). From these studies, it is clear that the number of mast cells in the upper dermis is increased in the electrohypersensitivity group. A different pattern of mast cell distribution also occurred in the electrohypersensitivity group, namely, the normally empty zone between the dermo-epidermal junction and mid-to-upper dermis disappeared in the electrohypersensitivity group and, instead, this zone had a high density of mast cell infiltration. These cells also seemed to have a tendency to migrate towards the epidermis (=epidermiotrophism) and many of them emptied their granular content (=degranulation) in the dermal papillary layer. Furthermore, more degranulated mast cells could be seen in the dermal reticular layer in the electrohypersensitivity group, especially in those cases which had the mast cell epidermiotrophism phenomenon described above. Finally, in the electrohypersensitivity group, the cytoplasmic granules were more densely distributed and more strongly stained than in the control group, and, generally, the size of the infiltrating mast cells was found to be larger in the electrohypersensitivity group as well. It should be noted, that increases of similar nature later on were demonstrated in an experimental situation employing normal healthy volunteers in front of visual display units, including ordinary house-hold television sets (cf. Johansson et al, 2001).

Mast cells, when activated, release a spectrum of mediators, among them histamine, which is involved in a variety of biological effects with clinical relevance, e.g., allergic hypersensitivity, itch, edema, local erythema, and many types of dermatoses. From the results of the above studies, it is clear that electromagnetic fields affect the mast cell, and also the dendritic cell, population, and may degranulate these cells.

The release of inflammatory substances, such as histamine, from mast cells in the skin results in a local erythema, edema, and sensation of itch and pain, and the release of somatostatin from the dendritic cells may give rise to subjective sensations of ongoing inflammation and sensitivity to ordinary light. These are, as mentioned, the common symptoms reported from persons suffering from electrohypersensitivity/screen dermatitis. Mast cells occur in the brain (Zhuang et al, 1999) and their presence may, under the influence of electromagnetic field and/or radiofrequency radiation exposure lead to chronic inflammatory response by the mast cell degranulation.

Mast cells are also present in the heart tissue and their localization is of particular relevance to their function. Data from studies made on interactions of electromagnetic fields with the cardiac function have demonstrated that changes are present in the heart after exposure to electromagnetic fields. Some electrically sensitive people have symptoms similar to heart attacks after exposure to electromagnetic fields.

We have also compared facial skin from electrohypersensitive persons with corresponding material from normal healthy volunteers (Johansson et al, 1996). The aim of the study was to evaluate possible markers to be used for future double-blind or blind provocation investigations. Differences were found for the biological markers calcitonin gene-related peptide (CGRP), somatostatin (SOM), vasoactive intestinal polypeptide (VIP), peptide histidine isoleucine amide (PHI), neuropeptide tyrosine (NPY), protein S-100 (S-100), neuron-specific enolase (NSE), protein gene product (PGP) 9.5 and phenylethanolamine N-methyltransferase (PNMT). The overall impression in the blind-coded material was such that it turned out easy to blindly separate the two groups from each other. However, no single marker was 100% able to pin-point the difference, although some were quite powerful in doing so (CGRP, SOM, S-100). In our on-going investigations, we have also found alterations of the Merkel cell number in the facial skin of electrohypersensitive persons (Yoshimura et al, 2006). However, it has to be pointed out that we cannot, based upon those results, draw any definitive conclusions about the cause of the changes observed. Blind or double-blind provocations in a controlled environment (Johansson et al, 2001) are necessary to elucidate the underlying causes for the changes reported in this particular investigation.

Gangi and Johansson (1997, 2000) have proposed models for how mast cells and substances secreted from them (e.g., histamine, heparin, and serotonin) could explain sensitivity to electromagnetic fields similar to those used to explain UV- and ionizing irradiation-related damages. We discuss an increasing number of persons who report cutaneous problems as well as symptoms from certain internal organs, such as the central nervous system and the heart, when being close to electric equipment. Many of these respondents are users of video display terminals, and have both subjective and objective skin- and mucosa-related symptoms, such as pain, itch, heat sensation, erythema, papules, and pustules. The central nervous system-derived symptoms are, e.g., dizziness, tiredness, and headache, erythema, itch, heat sensation, edema, and pain which are also common symptoms of sunburn (UV dermatitis). Alterations have been observed in cell populations of the skin of electrohypersensitive persons similar to those observed in the skin damaged due to ultraviolet light or ionizing radiation.

Gangi and Johansson (1997, 2000), have proposed a theoretical mechanism to explain how mast cells and substances secreted from them could cause sensitivity to electromagnetic fields. The mechanism derives from known facts in the fields of UV- and ionizing irradiation-related damage. Alterations seen after power-frequency or microwave electromagnetic field exposures that result in electrohypersensitivity symptoms may be understood by comparison to to ionizing radiation damage according to the type of immune function responses seen in both.

The working hypothesis is that electrohypersensitivity is a kind of irradiation damage, since the observed cellular changes are very much the same as the ones documented in tissue subjected to UV-light or ionizing radiation (see references below).

Mast cells are located in close proximity to neurons in the peripheral and central nervous systems, suggesting a functional role in normal and aberrant neurodegenerative states. They also possess many of the features of neurons, in terms of monoaminergic systems, responsiveness to neurotrophins and neuropeptides and the ability to synthesise and release bioactive neurotrophic factors. Mast cells are able to secrete an array of potent mediators which may orchestrate neuroinflammation and affect the integrity of the blood-brain barrier. The «cross-talk» between mast cells, lymphocytes, neurons and glia constitutes a neuroimmune axis which is implicated in a range of neurodegenerative diseases with an inflammatory and/or autoimmune component, such as multiple sclerosis and Alzheimer's disease.

Mast cells are involved in numerous activities ranging from control of the vasculature, to tissue injury and repair, allergic inflammation and host defences. They synthesize and secrete a variety of mediators, activating and modulating the functions of nearby cells and initiating complex physiological changes. Interestingly, NO produced by mast cells and/or other cells in the microenvironment appears to regulate these diverse roles. Some of the pathways central to the production of NO by mast cells and many of the tightly controlled regulatory mechanisms involved have been identified. Several cofactors and regulatory elements are involved in NO production, and these act at transcriptional and post-translational sites. Their involvement in NO production and the possibility that these pathways are critically important in mast cell functions should be investigated. The effects of NO on mast cell functions such as adhesion, activation and mediator secretion ought to be examined with a focus on molecular mechanisms by which NO modifies intracellular signalling pathways dependent or independent of cGMP and soluble guanylate cyclase. Metabolic products of NO including peroxynitrite and other reactive species may be the critical elements that affect the actions of

NO on mast cell functions. Further understanding of the actions of NO on mast cell activities may uncover novel strategies to modulate inflammatory conditions.

It is important to remember that mastocytosis - an abnormal accumulation of mast cells in one or more organ system - can occur secondarily to other causes, such as inflammation and some kinds of leukemia. The increase in EHS being described here is more accurately thought of as “primary” mastocytosis, meaning that the increased number of mast cells occurs independently of any other cause. However, because of the increased number of mast cells in primary mastocytosis, conditions such as osteoporosis and inflammation may arise as a result of the activity of those mast cells. The manner in which primary mastocytosis can be distinguished from secondary mastocytosis and other conditions should be addressed.

Research of mast cells and mastocytosis has made impressive progress over the past decade toward understanding what is different about mast cells in patients who have mastocytosis compared with mast cells in people who do not. A group of 23 researchers from Europe and the United States met in Vienna in September, 2000, and, after lengthy discussions, arrived at a consensus as to what criteria will accurately diagnose mastocytosis, and how to classify the various sub-types. Their conclusions are reported in a series of articles in the July, 2001, issue of Leukemia Research. Unfortunately, nothing was mentioned about mast cells and EMF effects.

Patients with mastocytosis may or may not have constitutional symptoms, including weight loss, pain, nausea, headache, malaise, or fatigue. These symptoms may be due to uncontrolled proliferation of mast cells or involvement of distinct organs, such as the stomach and intestines, or bone or bone marrow. Constitutional symptoms also can result from high levels of mast cell mediators in the blood stream. The severity of symptoms varies from mild to life-threatening.

The study of biopsy tissue in patients with suspected mastocytosis requires the use of appropriate stains. Tryptase is the stain of choice, as toluidine blue and Giemsa stains are more likely to be affected by tissue processing and may not always produce reliable results.

In skin, accumulation of groups of mast cells combined with the presence of urticaria pigmentosa or mastocytoma is diagnostic of cutaneous mastocytosis. In some cases, it may be difficult to establish a diagnosis. The absence of skin lesions does not rule out the diagnosis of mastocytosis.

The abnormalities that may be seen in mastocytosis mast cells are elongated shape, oval nuclei that are not in the center of the mast cell, and fewer than usual granules inside the mast cells, with those present being in groups rather than scattered. If two or more of these features are found, the cells are referred to as atypical mast cells. Sometimes the nucleus of atypical mast cells will have "lobes."

When the diagnosis of mastocytosis has not previously been established, specialized analyses may be required to differentiate between mastocytosis and other non-mast cell disorders of the blood-forming system, such as leukemias and myeloproliferative disorders. In some of these other disorders, the diseased cells contain and release low amounts of tryptase. Additional blood cell studies and chromosome analysis may be necessary to make a clear diagnosis in such cases.

Holmboe and Johansson (2005) reported on testing for the presence of increased levels of IgE or signs of a positive Phadiatop Combi (which is a screening test for allergies towards certain articles of food, pollen, insects, and other animals) which both would be indicators of an immune system alert. Twenty-two people (5 men, 17 women) participated in the study. Skin and nervous system effects were the primary symptoms reported by participants in the study. The most frequently reported symptoms were skin redness, eczema and sweating, loss of memory, concentration difficulties, sleep disturbances, dizziness, muscular and joint-related pain, and muscular and joint-related weakness. Headache, faintness, nasal stuffiness, and fatigue were also common. In addition, 19 of the people had disturbances of the gastrointestinal tract. All the people with the impairment electrohypersensitivity had tinnitus.

No connection between IgE blood levels and symptoms were found. All the people who reported electrohypersensitivity had normal values (<122 kU/l). Only 3 people had a positive Phadiatop Combi. Such increases could be used in the diagnosis of electrohypersensitivity, but they were not found to be useful indicators.

Animal Studies

In addition to the studies in humans, series of animal experiments were performed in collaboration with the Department of Biology, Faculty of Sciences, Novi Sad, Serbia and Montenegro), and the Karolinska Institute, Stockholm, Sweden (Rajkovic et al, 2005a,b, 2006).

The aim of these was to investigate the influence of extremely low-frequency electromagnetic fields (ELF-EMFs) on mast cells, parafollicular cells, and nerve fibers in rat skin and thyroid gland, as seen using light and transmission electron microscopy. The experiments were performed on 2-month-old Wistar male rats exposed for 4 h a day, 5 or 7 days a week for 1 month to power-frequent (50 Hz) EMFs (100-300 μT, 54-160 V/m). After sacrifice, samples of skin and thyroid were processed for indirect immunohistochemistry or toluidine blue staining and were then analyzed using the methods of stereology. Antibody markers to serotonin, substance P, calcitonin gene-related peptide (CGRP), and protein gene product 9.5 (PGP) were applied to skin sections and PGP, CGRP, and neuropeptide Y (NPY) markers to the thyroid. A significantly increased number of serotonin-positive mast cells in the skin ($p<0.05$) and NPY-containing nerve fibers in the thyroid ($p<0.01$) of rats exposed to ELF-EMF was found compared to controls, indicating a direct EMF effect on skin and thyroid vasculature.

After ultrastructural examination, a predominance of microfollicles with less colloid content and dilated blood capillaries was found in the EMF group. Stereological counting showed a statistically significant increase of the volume density of follicular epithelium, interfollicular tissue and blood capillaries as well as the thyroid activation index, as compared to the controls. The volume density of colloid significantly decreased. Ultrastructural analysis of thyroid follicular cells in the EMF group revealed the frequent finding of several colloid droplets within the same thyrocyte with the occasional presence of large-diameter droplets. Alterations in lysosomes, granular endoplasmic reticulum and cell nuclei compared to the control group were also observed. Taken together, the results of this study show the stimulative effect of power-frequency EMFs on thyroid gland at both the light microscope and the ultrastructural level.

The-animal results reported in these studies can not be explained away as psychosomatic in origin because they were conducted on animals, not humans.

In summary, both human and animal studies report large immunohistological changes in mast cells, and other measures of immune disfunction and disregulation due to exposures to ELF and RF at environmental levels associated with new electrical and wireless technologies.

It iss evident from our preliminary experimental data that various biological alterations are present in the electrohypersensitive persons claiming to suffer from exposure to electromagnetic fields. The alterations are themselves enough to fully explain the EHS symptoms, and the involvement of the immune system is evident. In view of recent epidemiological studies, pointing to a correlation between long-term exposure from power-frequent magnetic fields or microwaves and cancer, our data ought to be taken seriously and to be further analyzed.

Thus, it is of paramount importance to continue the investigation of persons with the impairment electrohypersensitivity. We would favour studies of electromagnetic fields' interaction with mast cell release of histamine and other biologically active substances, studies of lymphocyte viability as well as studies of the newly described serotonin-containing melanocytes. Also, continued analysis of the intraepidermal nerve fibers and their relations to these mast cells and serotonin-containing melanocytes are very important. Finally, not to be forgotten, a general investigation - of persons with the impairment electrohypersensitivity versus normal healthy volunteers - regarding the above markers as well as other markers for cell traffic, proliferation and inflammation is very much needed. Such scientific work may lay a firm foundation for necessary adjustment of accessibility, thus helping and supporting all persons with the functional impairment electrohypersensitivity.

VI. Direct effects of EMFs on the immune system

Childhood leukemia was early connected to power-frequent magnetic fields already in the pioneering work by Wertheimer and Leeper (1979), and more recently Scandinavian scientists have identified an increased risk for acoustic neuroma (i.e., a benign tumor of the eighth cranial nerve) in cell phone users, as well as a slightly increased risk of malignant brain tumors such as astrocytoma and meningioma on the same side of the brain as the cell phone was habitually held (Hardell et al, 1999, 2004, 2005; Lonn et al, 2004). In addition, a clear association between adult cancers and FM radio broadcasting radiation has been noticed, both in time and location (Hallberg and Johansson, 2002b, 2004a, 2005a). Initial studies on facial nevi indicates that nowadays also young children can have a substantial amount of these. If it can be shown that radiofrequency radiation is not correlated with childhood cancers the current focus on low-frequency electromagnetic fields can continue. If there is also a radiofrequency and/or microwave correlation then this must be considered in future research as well as in today's preventive work.

Anane and coworkers (2003) studied the effects of acute exposure to GSM-900 microwaves (900 MHz, 217 Hz pulse modulation) on the clinical parameters of the acute experimental allergic encephalomyelitis (EAE) model in rats in two independent experiments: rats were either habituated or nonhabituated to the exposure restrainers. EAE was induced with a mixture of myelin basic protein and Mycobacterium tuberculosis. Female Lewis rats were divided into cage control, sham exposed, and two groups exposed either at 1.5 or 6.0 W/kg local specific absorption rate (SAR averaged over the brain) using a loop antenna placed over their heads. No effect of a 21-day exposure (2 h/day) on the onset, duration, and termination of the EAE crisis was seen.

The object of the study by Boscol et al. (2001) was to investigate the immune system of 19 women with a mean age of 35 years, for at least 2 years (mean = 13 years) exposed to electromagnetic fields induced by radiotelevision broadcasting stations in their residential area. In September 1999, the EMFs (with range 500 KHz-3 GHz) in the balconies of the homes of the women were (mean +/- S.D.) 4.3 +/- 1.4 V/m. Forty-seven women of similar age, smoking habits and atopy composed the control group, with a nearby resident EMF exposure of < 1.8 V/m. Blood lead and urinary trans-trans muconic acid (a metabolite of benzene), markers of exposure to urban traffic, were higher in the control women. The EMF exposed group showed a statistically significant reduction of blood NK CD16+-CD56+, cytotoxic CD3(-)-CD8+, B and NK activated CD3(-)-HLA-DR+ and CD3(-)-CD25+ lymphocytes. 'In vitro' production of IL-2 and interferon-gamma (INF-gamma) by peripheral blood mononuclear cells (PBMC) of the EMF exposed group, incubated either with or without phytohaemoagglutinin (PHA), was significantly lower; the 'in vitro' production of IL-2 was significantly correlated with blood CD16+-CD56+ lymphocytes. The stimulation index (S.I.) of blastogenesis (ratio between cell proliferation with and without PHA) of PBMC of EMF exposed women was lower than that of the control subjects. The S.I. of blastogenesis of the EMF exposed group (but not blood NK lymphocytes and the 'in vitro' production of IL-2 and INF-gamma by PBMC) was significantly correlated with the EMF levels. Blood lead and urinary trans-trans muconic acid were barely correlated with immune parameters: the urinary metabolite of benzene of the control group was only correlated with CD16+-CD56+ cells indicating a slight effect of traffic on the immune system. In conclusion, this study demonstrates that high-frequency EMFs reduce cytotoxic activity in the peripheral blood of women without a dose-response effect. Such an effect could, of course, only be considered as very serious, since this could hamper the immune system in it's daily struggle against various organisms/agents.

On the other hand, Chagnaud and Veyret in 1999 could not demonstrate an effect of low-level pulsed microwaves on the integrity of the immune system. They investigated the effects of GSM-modulated microwaves on lymphocyte sub-populations of Sprague-Dawley rats and their normal mitogenic responses using flow cytometry analysis and a colorimetric method. No alterations were found in the surface phenotype of splenic lymphocytes or in their mitogenic activity.

Cleary et al. (1990) reported a biphasic, dose-dependent effect of microwave radiation on lymphycyte proliferation with non-thermal exposures. Whole human blood was exposed or sham-exposed in vitro for 2 h to 27 or 2,450 MHz radio-frequency electromagnetic (RF) radiation under isothermal conditions (i.e., 37 +/- 0.2 degrees C). Immediately after exposure, mononuclear cells were separated from blood by Ficoll density-gradient centrifugation and cultured for 3 days at 37 degrees C with or without mitogenic stimulation by phytohemagglutinin (PHA). Lymphocyte proliferation was assayed at the end of the culture period by 6 h of pulse-labeling with 3H-thymidine (3H-TdR). Exposure to radiation at either frequency at specific absorption rates (SARs) below 50 W/kg resulted in a dose-dependent, statistically significant increase of 3H-TdR uptake in PHA-activated or unstimulated lymphocytes. Exposure at 50 W/kg or higher suppressed 3H-TdR uptake relative to that of sham-exposed cells. There were no detectable effects of RF radiation on lymphocyte morphology or viability. Notwithstanding the characteristic temperature dependence of lymphocyte activation in vitro, the isothermal exposure conditions of this study warrant the conclusion that the biphasic, dose-dependent effects of the radiation on lymphocyte proliferation were not dependent on heating.

Cleary et al. (1996) subsequently published ~~yet~~ another paper reporting a biphasic response of lymphycytes to radiofrequency/microwave radiation where higher SARs resulted in decreased cell proliferation and lower SARs result in increased cell proliferation, dependent on the mitotic state of the cells. –Previous in vitro studies had provided evidence that RF electromagnetic radiation modulates proliferation of human glioma, lymphocytes, and other cell types. The mechanism of such RF radiation cell proliferation modulation, as well as mechanisms for effects on other cell physiologic endpoints, however, were not well understood. To obtain insight regarding interaction mechanisms, they investigated effects of RF radiation exposure on interleukin 2 (IL-2) -dependent proliferation of cytolytic T lymphocytes (CTLL-2). After exposure to RF radiation in the presence or absence of IL-2 cells were cultured at various physiological concentrations of IL-2. Treatment effects on CTLL-2 proliferation were determined by tritiated thymidine incorporation immediately or 24 h after exposure. Exposure to 2,450 MHz RF radiation at specific absorption rates (SARs) of greater than 25 W/kg (induced E-field strength 98.4 V/m) induced a consistent, statistically significant reduction in CTLL-2 proliferation, especially at low IL-2 concentrations. At lower SARs, 2,450 MHz exposure increased CTLL-2 proliferation immediately after exposure but reduced 24 h post-exposure proliferation. RF radiation effects depended on the mitotic state of the cells at the time of exposure.

In 1992, Czerska et al. studied the effects of continuous and pulsed 2,450-MHz radiation on spontaneous lymphoblastoid transformation of human lymphocytes in vitro. Normal human lymphocytes were isolated from the peripheral blood of healthy donors. One-ml samples containing one million cells in chromosome medium 1A were exposed for 5 days to conventional heating or to continuous wave (CW) or pulsed wave (PW) 2,450-MHz radiation at non-heating (37 degrees C) and various heating levels (temperature increases of 0.5, 1.0, 1.5, and 2 degrees C). The pulsed exposures involved 1-microsecond pulses at pulse repetition frequencies from 100 to 1,000 pulses per second at the same average SAR levels as the CW exposures. Actual average SARs ranged to 12.3 W/kg. Following termination of the incubation period, spontaneous lymphoblastoid transformation was determined with an image analysis system. The results were compared among each of the experimental conditions and with sham-exposed cultures. At non-heating levels, CW exposure did not affect transformation. At heating levels both conventional and CW heating enhanced transformation to the same extent and correlate with the increases in incubation temperature. PW exposure enhanced transformation at non-heating levels. This finding is significant ($p<0.002$). At heating levels PW exposure enhanced transformation to a greater extent than did conventional or CW heating. This finding is significant at the 0.02 level. It was concluded that PW 2,450-MHz radiation acts differently on the process of lymphoblastoid transformation in vitro compared with CW 2,450-MHz radiation at the same average SARs.

In 2003, Dabrowski et al. exposed samples of mononuclear cells isolated from peripheral blood of healthy donors ($n = 16$) to 1,300 MHz pulse-modulated microwaves at 330 pps with 5 μs pulse width. The samples were exposed in an anechoic chamber at the average value of power density of $S = 10$ W/m^2 (1 mW/cm^2). The average specific absorption rate (SAR) was measured in rectangular waveguide and the value of SAR = 0.18 W/kg was recorded. Subsequently, the exposed and control cells were assessed in the microculture system for several parameters characterizing their proliferative and immunoregulatory properties. Although the irradiation decreased the spontaneous incorporation of 3H-thymidine, the proliferative response of lymphocytes to phytohemagglutinin (PHA) and to Con A as well as the T-cell suppressive activity (SAT index) and the saturation of IL-2 receptors did not change. Nevertheless, the lymphocyte production of interleukin (IL)-10 increased ($p< 0.001$) and the concentration of IFNγ remained unchanged or slightly decreased in the culture supernatants. Concomitantly, the microwave irradiation modulated the monokine production by monocytes. The production of IL-1β increased significantly ($p< 0.01$), the concentration of its antagonist (IL-1ra) dropped by half ($p< 0.01$) and the tumor necrosis factor (TNF-α) concentration remained unchanged. These changes of monokine proportion (IL-1 β vs. IL-1ra) resulted in significant increase of the value of LM index ($p<0.01$), which reflects the activation of monocyte immunogenic function. The results indicate that pulse-modulated microwaves represent the potential of immunotropic influence, stimulating preferentially the immunogenic and proinflammatory activity of monocytes at relatively low levels of exposure,

Following these findings of G_o phase peripheral blood mononulclear cells (PBMC) exposed to low-level (SAR = 0.18 W/kg) pulse-modulated 1300 MHz microwave,s and subsequently cultured, demonstrating changed immune activity (as of above), in 2006 Stankiewicz and coworkers investigated whether cultured immune cells induced into the active phases of cell cycle (G_1, S) and then exposed to microwaves will also be sensitive to electromagnetic fields. An anechoic chamber containing a microplate with cultured cells and an antenna emitting microwaves (900 MHz simulated GSM signal, 27 V/m, SAR 0.024 W/kg) was placed inside an ASSAB incubator. The microcultures of PBMC exposed to microwaves demonstrated significantly higher response to mitogens and higher immunogenic activity of monocytes (LM index) than control cultures. The LM index, described in detail elsewhere (Dabrowski et al, 2001), represents the monokine influence on lymphocyte mitogenic response. The results suggest that immune activity of responding lymphocytes and monocytes can be additionally intensified by 900 MHz microwaves. The above described effects of an immune system activity-intensifying effect of 900 MHz microwaves are, of course, a very important warning signal as well as a very important piece of the explanatory jigsaw puzzle regarding, for instance, the functional impairment electrohypersensitivity. In the latter, affected persons very often describe “influenza-like” sensations in their body. Maybe the mobile phones, as well as other high-frequency devices, have aroused the immune system to a too high an activation level?

In an attempt to understand how non-atopic and atopic fertile women with uniform exposure to toxic compounds produced by traffic - immunologically react to high or low frequency electromagnetic fields (ELMF), Del Signore et al. (2000) performed a preliminary study. Women were divided in group A (non-atopic, non-exposed to ELMF); B (atopic, non-exposed to ELMF); C (non-atopic, exposed to ELMF); D (atopic, exposed to ELMF). In vitro cell proliferation of peripheral blood mononuclear cells (PBMC) of atopic women (groups B and D) stimulated by phytohaemoglutinin (PHA) was reduced. The ELMF exposed women (groups C and D) showed lower levels of blood NK CD16(+)-CD56+ lymphocyte subpopulations and of "in vitro" production of interferon-gamma (both spontaneously and in presence of PHA) by PBMC, suggesting that ELMF reduces blood cytotoxic activity. Serum IgE of the atopic women exposed to ELMF (group D) was higher than that of the other groups. Linear discriminant analysis including serum zinc and copper (essential enzymes for immune functions), blood lead and urinary transtrans muconic acid, a metabolite of benzene (markers of exposure to traffic) and key parameters of immune functions (CD16(+)-CD56+ lymphocyte subset, serum IgE, interferon-gamma produced by PBMC in presence of PHA, stimulation index of blastogenesis) showed absence of significant difference between groups A and C and a marked separation of groups B and D. This datum suggests that ELMF have a greater influence on atopic women exposed to traffic than on non-atopic ones, again pointing out differing reaction capacities in the human population – maybe dependent on varying immune functions based on variations in genetic make-up.

A more general reaction pattern was found by Dmoch and Moszczynski (1998) who assessed immunoglobulin concentrations and T-lymphocyte subsets in workers of TV re-transmission and satellite communication centres. An increase in IgG and IgA concentrations, an increased count of lymphocytes and T8 lymphocytes, an decreased count of NK cells and a lower value of T-helper/T-suppressor ratio were found.

Elekes et al. (1996) found a very interesting sex-difference. The effect of continuous (CW; 2.45 GHz carrier frequency) or amplitude-modulated (AM; 50 Hz square wave) microwave radiation on the immune response was tested. CW exposures (6 days, 3 h/day) induced elevations of the number of antibody-producing cells in the spleen of male Balb/c mice (+37%). AM microwave exposure induced elevation of the spleen index (+15%) and antibody-producing cell number (+55%) in the spleen of male mice. No changes were observed in female mice. It is concluded that both types of exposure conditions induced moderate elevation of antibody production only in male mice.

Irradiation with electromagnetic waves (8.15-18 GHz, 1 Hz within, 1 microW/cm2) in vivo increases the cytotoxic activity of natural killer cells of rat spleen (Fesenko et al, 1999a). In mice exposed for 24-72 h, the activity of natural killer cells increased by 130-150%, the increased level of activity persisting within 24 h after the cessation of treatment. Microwave irradiation of animals in vivo for 3.5 and 5 h, and a short exposure of splenic cells in vitro did not affect the activity of natural killer cells.

Whole body microwave sinusoidal irradiation of male NMRI mice with 8.15-18 GHz (1 Hz within) at a power density of 1 microW/cm2 caused a significant enhancement of TNF production in peritoneal macrophages and splenic T lymphocytes (Fesenko et al, 1999b). Microwave radiation affected T cells, facilitating their capacity to proliferate in response to mitogenic stimulation. The exposure duration necessary for the stimulation of cellular immunity ranged from 5 h to 3 days. Chronic irradiation of mice for 7 days produced the decreasing of TNF production in peritoneal macrophages. The exposure of mice for 24 h increased the TNF production and immune proliferative response, and these stimulatory effects persisted over 3 days after the termination of exposure. Microwave treatment increased the endogenously produced

TNF more effectively than did lipopolysaccharide, one of the most potential stimuli of synthesis of this cytokine. Microwaves, thus, indeed can be a factor interfering with the process of cell immunity!

Gapeev et al. (1996) reported that low-intensity electromagnetic radiation of extremely high frequency in the near field of modified the acitivity of mouse peritoneal neutrophils in a quasi-reasonance fashion., He compared the effect of radiation from various types of antennae, including one which created a uniform spatial distribution of specific absorbed rating in the frequency range used and wide-band matching with the object both in near field and far field zones of the radiator. The authors extremely high frequency in near field zone but not the far field zone of the channel radiator modified the activity of mouse peritoneal neutrophils on a quasi-resonance manner. The interaction of electromagnetic radiation with the biological object has been revealed in the narrow-band frequencies of 41.8-42.05 GHz and consists in inhibition of luminol-dependent chemiluminescence of neutrophils activated by opsonized zymosan. It is not found any frequency dependence of the electromagnetic radiation effects in the far field zone of the radiator. The results obtained suggest, that the quasi-resonance dependence of the biological effect on the frequency of the electromagnetic radiation in the near field zone is conditioned by structure and nature of the electromagnetic radiation in this zone.

In 2003, Gatta et al. studied the effects of in vivo exposure to GSM-modulated 900 MHz radiation on mouse peripheral lymphocytes. The aim of this study was to evaluate whether daily whole-body exposure to 900 MHz GSM-modulated radiation could affect spleen lymphocytes. C57BL/6 mice were exposed 2 h/day for 1, 2 or 4 weeks in a TEM cell to an SAR of 1 or 2 W/kg. Untreated and sham-exposed groups were also examined. At the end of the exposure, mice were killed humanely and spleen cells were collected. The number of spleen cells, the percentages of B and T cells, and the distribution of T-cell subpopulations (CD4 and CD8) were not altered by the exposure. T and B cells were also stimulated ex vivo using specific monoclonal antibodies or LPS to induce cell proliferation, cytokine production and expression of activation markers. The results did not show relevant differences in either T or B lymphocytes from mice exposed to an SAR of 1 or 2 W/kg and sham-exposed mice with few exceptions. After 1 week of exposure to 1 or 2 W/kg, an increase in IFN-gamma (Ifng) production was observed that was not evident when the exposure was prolonged to 2 or 4 weeks. This suggests that the immune system might have adapted (!) to RF radiation as it does with other stressing agents. All together, from their in vivo data, they made the conclusion that it indicated that the T- and B-cell compartments were not substantially affected by exposure to RF radiation and that a clinically relevant effect of RF radiation on the immune system is unlikely to occur. Another explanation could be that the cells were unable to deal with the exposure and the obvious follow-up question then will be: What happened with the immune cells after months and years of exposure?

On the other hand, Kolomytseva et al. (2002), in their whole-body exposure experiment designed to study the dynamics of leukocyte number and functional activity of peripheral blood neutrophils under whole-body exposure of healthy mice to low-intensity extremely-high-frequency electromagnetic radiation (EHF EMR, 42.0 GHz, 0.15 mW/cm2, 20 min daily), showed that such a whole-body exposure of healthy mice to low-intensity EHF EMR has a profound effect on the indices of nonspecific immunity. It was shown that the phagocytic activity of peripheral blood neutrophils was suppressed by about 50% ($p<0.01$ as compared with the sham-exposed control) in 2-3 h after the single exposure to EHF EMR. The effect persisted for 1 day after the exposure, and then the phagocytic activity of neutrophils returned to the norm within 3 days. A significant modification of the leukocyte blood profile in mice exposed to EHF EMR for 5 days was observed after the cessation of exposures: the number of leukocytes increased by 44% ($p<0.05$ as compared with sham-exposed animals), mostly due to an increase in the lymphocyte content. The supposition was made that EHF EMR effects can be mediated via the metabolic systems of arachidonic acid and the stimulation of adenylate cyclase activity, with subsequent increase in the intracellular cAMP level.

The modification of indices of the humoral immune response to thymus-dependent antigen (sheep erythrocytes) after a whole-body exposure of healthy mice to low-intensity extremely-high-frequency electromagnetic radiation was reported by Lushnikov et al. in 2001. Male NMRI mice were exposed in the far-field zone of horn antenna at a frequency of 42.0 GHz and energy flux density of 0.15 mW/cm2 under different regimes: once for 20 min, for 20 min daily during 5 and 20 successive days before immunization, and for 20 min daily during 5 successive days after immunization throughout the development of the humoral immune response. The intensity of the humoral immune response was estimated on day 5 after immunization by the number of antibody-forming cells of the spleen and antibody titers. Changes in cellularity of the spleen, thymus and red bone marrow were also assessed. The indices of humoral immunity and cellularity of lymphoid organs changed insignificantly after acute exposure and series of 5 exposures before and after immunization of the animals. However, after repeated exposures for 20 days before immunization, a statistically significant reduction of thymic cellularity by 17.5% ($p<0.05$) and a decrease in cellularity of the spleen by 14.5% ($p<0.05$) were revealed. The results show that low-intensity extremely-high-frequency electromagnetic radiation with the frequency and energy flux density used does not influence the humoral immune response intensity in healthy mice but influences immunogenesis under multiple repeated exposures.

The immunoglobulins' concentrations and T lymphocyte subsets during occupational exposures to microwave radiation were assessed in 1999 by Moszczynski et al. In the workers of retransmission TV center and center of satellite communications on increased IgG and IgA concentration and decreased count of lymphocytes and T8 cells was found. However, in the radar operators IgM concentration was elevated and a decrease in the total T8 cell count was observed. The different behaviour of examined immunological parameters indicate that the effect of microwave radiation on immune system depends on character of an exposure. Disorders in the immunoglobulins' concentrations and in the T8 cell count did not cause any reported clinical consequences.

Experiments have also been conducted to elucidate the effects of chronic low power-level microwave radiation on the immunological systems of rabbits (Nageswari et al, 1991). Fourteen male Belgian white rabbits were exposed to microwave radiation at 5 mW/cm2, 2.1 GHz, 3 h daily, 6 days/week for 3 months in two batches of 7 each in specially designed miniature anechoic chambers. Seven rabbits were subjected to sham exposure for identical duration. The microwave energy was provided through S band standard gain horns connected to a 4K3SJ2 Klystron power amplifier. The first batch of animals were assessed for T lymphocyte-mediated cellular immune response mechanisms and the second batch of animals for B lymphocyte-mediated humoral immune response mechanisms. The peripheral blood samples collected monthly during microwave/sham exposure and during follow-up (5/14 days after termination of exposures, in the second batch animals only) were analysed for T lymphocyte numbers and their mitogen responsiveness to ConA and PHA. Significant suppression of T lymphocyte numbers was noted in the microwave group at 2 months (p less than 0.01) and during follow-up (p less than 0.01). The first batch animals were initially sensitised with BCG and challenged with tuberculin (0.03 ml) at the termination of microwave irradiation/sham exposure and the increase in foot pad thickness (delta mm), which is a measure of T cell-mediated immunity (delayed type hypersensitivity response, DTH) was noted in both the groups. The microwave group revealed a more robust response than the control group (delta % +12.4 vs. +7.54).

Nakamura et al. (1997) reported on the effect of microwaves on pregnant rats. The authors reported that microwaves at the power of 10 mW/cm2 produced activation of the hypothalamic-pituitary-adrenal axis and increased oestradiol in both virgin and pregnant rats, suggesting that microwaves greatly stress pregnant organisms. Earlier data had indicated that these microwaves produce various detrimental changes based on actions of heat or non-specific stress, although the effects of microwaves on pregnant organisms was not uniform. This study was therefore designed to clarify the effect of exposure to microwaves during pregnancy on endocrine and immune functions. Natural killer cell activity and natural killer cell subsets in the spleen were measured, as well as some endocrine indicators in blood--corticosterone and adrenocorticotrophic hormone (ACTH) as indices of the hypothalamic-pituitary-adrenal axis--beta-endorphin, oestradiol, and progesterone in six female virgin rats and six pregnant rats (nine to 11 days gestation) exposed to microwaves at 10 mW/cm2 incident power density at 2,450 MHz for 90 minutes. The same measurements were performed in control rats (six virgin and six pregnant rats). Skin temperature in virgin and pregnant rats increased immediately after exposure to microwaves. Although splenic activity of natural killer cells and any of the subset populations identified by the monoclonal antibodies CD16 and CD57 did not differ in virgin rats with or without exposure to microwaves, pregnant rats exposed to microwaves showed a significant reduction of splenic activity of natural killer cells and CD16+CD57-. Although corticosterone and ACTH increased, and oestradiol decreased in exposed virgin and pregnant rats, microwaves produced significant increases in beta-endorphin and progesterone only in pregnant rats.

Nakamura et al. (1998) evaluated the involvement of opioid systems in reduced natural killer cell activity (NKCA) in pregnant rats exposed to microwaves at a relatively low level (2 mW/cm2 incident power density at 2,450 MHz for 90 min). They assayed beta-endorphin (betaEP) in blood, pituitary lobes, and placenta as well as splenic NKCA in virgin and/or pregnant rats. Although microwaves elevated colonic temperatures by 0.8 degrees C for virgin and 0.9 degrees C for pregnant rats, and betaEP in blood and anterior pituitary lobes (AP) significantly, it did not change blood corticosterone as an index of hypothalamic-pituitary adrenal axis. There were significant interactions between pregnancy and microwave exposure on splenic NKCA, betaEP in both blood and AP, and blood progesterone. Intraperitoneal administration of opioid receptor antagonist naloxone prior to microwave exposure increased NKCA, blood, and placental betaEP in pregnant rats. Alterations in splenic NKCA, betaEP and progesterone in pregnant rats exposed to microwaves may be due to both thermal and non-thermal actions. These results suggest that NKCA reduced by microwaves during pregnancy is mediated by the pituitary opioid system.

To further clarify the effects of microwaves on pregnancy, Nakamura et al. (2000) investigated rats exposed to continuous-wave (CW) microwave at 2 mW/cm(2) incident power density at 2,450 MHz for 90 min.. The effects on uterine or uteroplacental blood flow and endocrine and biochemical mediators, including corticosterone, estradiol, prostaglandin E(2) (PGE(2)), and prostaglandin F(2)alpha (PGF(2)alpha) were measured, Colonic temperature in virgin and pregnant rats was not significantly altered by microwave treatment. Microwaves decreased uteroplacental blood flow and increased progesterone and PGF(2)alpha in pregnant, but not in virgin rats. Intraperitoneal (i.p.) administration of angiotensin II, a uteroplacental vasodilator, before microwave exposure prevented the reduction in uteroplacental blood flow and the increased progesterone and PGF(2)alpha in pregnant rats. Increased corticosterone and decreased estradiol during microwave exposure were observed independent of pregnancy and pretreatment with angiotensin II. These results suggest that microwaves (CW, 2 mW/cm(2), 2,450 MHz) produce uteroplacental circulatory disturbances and ovarian and placental dysfunction during pregnancy, probably through non-thermal actions. The uteroplacental disturbances appear to be due to actions of PGF(2)alpha and may pose some risk for pregnancy. Reported pregnancy losses in women (Lee, 2001; Li, 2001) and infertility (Magras and Xenos, 1997) might be related to these laboratory findings.

Nasta et al. (2006), very recently examined the effects of in vivo exposure to a GSM-modulated 900 MHz RF field on B-cell peripheral differentiation and antibody production in mice. Their results show that exposure to a whole-body average specific absorption rate (SAR) of 2 W/kg, 2 h/day for 4 consecutive weeks does not affect the frequencies of differentiating transitional 1 (T1) and T2 B cells or those of mature follicular B and marginal zone B cells in the spleen. IgM and IgG serum levels are also not significantly different among exposed, sham-exposed and control mice. B cells from these mice, challenged in vitro with LPS, produce comparable amounts of IgM and IgG. Moreover, exposure of immunized mice to RF fields does not change the antigen-specific antibody serum level. Interestingly, not only the production of antigen-specific IgM but also that of IgG (which requires T-B-cell interaction) is not affected by RF-field exposure. This indicates that the exposure does not alter an ongoing in vivo antigen-specific immune response. In conclusion, the results of Nasta et al. (2006) do not indicate any effects of GSM-modulated RF radiation on the B-cell peripheral compartment and antibody production.

Whole-body microwave sinusoidal irradiation of male NMRI mice, exposure of macrophages in vitro, and preliminary irradiation of culture medium with 8.15-18 GHz (1 Hz within) at a power density of 1 microW/cm2 caused a significant enhancement of tumor necrosis factor production in peritoneal macrophages (Novoselova et al, 1998). The role of microwaves as a factor interfering with the process of cell immunity must, thus, be seriously considered. Furthermore the effect of 8.15-18 GHz (1 Hz within) microwave radiation at a power density of 1 microW/cm2 on the tumor necrosis factor (TNF) production and immune response was tested by Novoselova et al. (1999). A single 5 h whole-body exposure induced a significant increase in TNF production in peritoneal macrophages and splenic T cells. The mitogenic response in T lymphocytes increased after microwave exposure. The activation of cellular immunity was observed within 3 days after exposure. The diet containing lipid-soluble nutrients (beta-carotene, alpha-tocopherol and ubiquinone Q9) increased the activity of macrophages and T cells from irradiated mice.

Obukhan (1998) has performed cytologic investigations designed to study bone marrow, peripheral blood, spleen, and thymus of albino rats irradiated by an electromagnetic field, 2,375, 2,450, and 3,000 MHz. Structural and functional changes in populations of megakaryocytes, immunocompetent cells as well as of undifferentiated cells, and of other types of cells that are dependent on the intensity of irradiation.

The possibility of genotoxicity of radiofrequency radiation (RFR) applied alone or in combination with x-rays was recently investigated in vitro using several assays on human lymphocytes by Stronati and colleagues (2006). The chosen specific absorption rate (SAR) values are near the upper limit of actual energy absorption in localized tissue when persons use some cellular telephones. The purpose of the combined exposures was to examine whether RFR might act epigenetically by reducing the fidelity of repair of DNA damage caused by a well-characterized and established mutagen. Blood specimens from 14 donors were exposed continuously for 24 h to a Global System for Mobile Communications (GSM) basic 935 MHz signal. The signal was applied at two SAR; 1 and 2 W/Kg, alone or combined with a 1-min exposure to 1.0 Gy of 250 kVp x-rays given immediately before or after the RFR. The assays employed were the alkaline comet technique to detect DNA strand breakage, metaphase analyses to detect unstable chromosomal aberrations and sister chromatid exchanges, micronuclei in cytokinesis-blocked binucleate lymphocytes and the nuclear division index to detect alterations in the speed of in vitro cell cycling. By comparison with appropriate sham-exposed and control samples, no effect of RFR alone could be found for any of the assay endpoints. In addition RFR did not modify any measured effects of the x-radiation. In conclusion, this study has used several standard in vitro tests for chromosomal and DNA damage in Go human lymphocytes exposed in vitro to a combination of x-rays and RFR. It has comprehensively examined whether a 24-h continuous exposure to a 935 MHz GSM basic signal delivering SAR of 1 or 2 W/Kg is genotoxic per se or whether, it can influence the genotoxicity of the well-established clastogenic agent; x-radiation. Within the experimental parameters of the study in all instances no effect from the RFR signal was observed.

Tuschl et al. (1999) recorded a considerable excess of recommended exposure limits in the vicinity of shortwave diathermy devices used for medical treatment of patients. Different kinds of field probes were used to measure electric and magnetic field strength and the whole body exposure of medical personnel operating shortwave, decimeter wave and microwave units was calculated. To investigate the influence of chronic exposure on the immune system of operators, blood was sampled from physiotherapists working at the above mentioned devices. Eighteen exposed and thirteen control persons, matched by sex and age, were examined. Total leucocyte and lymphocyte counts were performed and leucocytic subpopulations determined by flow cytometry and monoclonal antibodies against surface antigens. In addition, to quantify subpopulations of immunocompetent cells, the activity of lymphocytes was measured. Lymphocytes were stimulated by mitogen phytohemagglutinin and their proliferation measured by a flow cytometric method. No statistically significant differences between the control and exposed persons were found. In both study groups all immune parameters were within normal ranges.

Despite the important role of the immune system in defending the body against infections and cancer, only few investigations on possible effects of radiofrequency (RF) radiation on function of human immune cells have been undertaken. One of these is the investigation by Tuschl et al. in 2005 where they assessed whether GSM modulated RF fields have adverse effects on the functional competence of human immune cells. Within the frame of the multidisciplinary project "Biological effects of high frequency electromagnetic fields (EMF)" sponsored by the National Occupation Hazard Insurance Association (AUVA) in vitro investigations were carried out on human blood cells. Exposure was performed at GSM Basic 1950 MHz, an SAR of 1 mW/g in an intermittent mode (5 min "ON", 10 min "OFF") and a maximum Delta T of 0.06 degrees C for the duration of 8 h. The following immune parameters were evaluated: (1) the intracellular production of interleukin-2 (IL-2) and interferon (INF) gamma in lymphocytes, and IL-1 and tumor necrosis factor (TNF)-alpha in monocytes were evaluated with monoclonal antibodies. (2) The activity of immune-relevant genes (IL 1-alpha and beta, IL-2, IL-2-receptor, IL-4, macrophage colony stimulating factor (MCSF)-receptor, TNF-alpha, TNF-alpha-receptor) and housekeeping genes was analyzed with real time PCR. (3) The cytotoxicity of lymphokine activated killer cells (LAK cells) against a tumor cell line was determined in a flow cytometric test. For each parameter, blood samples of at least 15 donors were evaluated. No statistically significant effects of exposure were found and there is no indication that emissions from mobile phones are associated with adverse effects on the human immune system.

Irradiation by pulsed microwaves (9.4 GHz, 1 microsecond pulses at 1,000/s), both with and without concurrent amplitude modulation (AM) by a sinusoid at discrete frequencies between 14 and 41 MHz, was assessed for effects on the immune system of Balb/C mice (Veyret et al, 1991). The mice were immunized either by sheep red blood cells (SRBC) or by glutaric-anhydride conjugated bovine serum albumin (GA-BSA), then exposed to the microwaves at a low rms power density (30 microW/cm2; whole-body-averaged SAR approximately 0.015 W/kg). Sham exposure or microwave irradiation took place during each of five contiguous days, 10 h/day. The antibody response was evaluated by the plaque-forming cell assay (SRBC experiment) or by the titration of IgM and IgG antibodies (GA-BSA experiment). In the absence of AM, the pulsed field did not greatly alter immune responsiveness. In contrast, exposure to the field under the combined-modulation condition resulted in significant, AM-frequency-dependent augmentation or weakening of immune responses.

Finally, in addition, classical allergy reactions, such as chromate allergy, has been studied by Seishima et al. (2003). The background for the study was an earlier case report about a patient with allergic contact dermatitis caused by hexavalent chromium plating on a cellular phone. The new study described the clinical characteristics and results of patch tests (closed patch tests and photopatch tests were performed using metal standard antigens) in 8 patients with contact dermatitis possibly caused by handling a cellular phone. The 8 patients were 4 males and 4 females aged from 14 to 54 years. They each noticed skin eruptions after 9-25 days of using a cellular phone. All patients had erythema, and 7 had papules on the hemilateral auricle or in the preauricular region. Three of 8 patients had a history of metal allergy. Chromate, aluminium and acrylnitrile-butadiene-styrene copolymer were used as plating on the cellular phones used by these patients. The patch test was positive for 0.5, 0.1 and 0.05% potassium dichromate in all 8 patients. The photopatch test showed the same results. One patient was positive for 2% cobalt chloride and one for 5% nickel sulfate. Based on these data, it is important to consider the possibility of contact dermatitis due to a cellular phone, possibly caused by chromate, when the patients have erythema and papules on the hemilateral auricle or in the preauricular region.

VII. Electromagnetic fields and health

Since the formation of life on Earth, as we know it, more than 3.5 billion years ago, the only real source of radiation, apart from Earth's static geomagnetic field, has been the sun. All living organisms that have evolved and not been able to cope with it are either gone or have adapted to it in one of several ways. Living under-ground, only being active during night, living in the deeper waters (1 meter or deeper) in oceans and lakes, under the foliage of jungle-trees, or - as all day-active organisms have – developed a skin (or, for plants, a cortex) containing a pigment (animals and plants have very similar ones) that will shield some heat and some sunshine…but not very much. Any fair-skinned Irish or Scandinavian person learns very early to avoid even the rather bleak sun up-north, because – if not – you will easily get a nasty sunburn. Later on, that sunburn will develop into a postinflammatory hyperpigmentation, with it's cosmetic values, however, well before it you will get a strong alarm signal in the form of a redness of the skin.

When considering other frequencies, the pigment does not furnish any protection at all, something mankind has found out during the last 100 years. Cosmic rays, radioactivity, X-rays, UVC, UVB and now even UVA are considered, together with radar-type microwaves to be very, or even extremely, dangerous to your health. You are translucent to exposures such as power-frequent magnetic fields as well as mobile phone and WI-FI microwaves, but this does not mean that they are without possible effect, through thermal or non-thermal mechanisms.

Is it possible that we can adapt our biology to altered exposure conditions in less than 100 years, or do we have to have thousands of years for such an adaptation? And, in the meantime, what kind of safety standards must we adopt if the current public safety limits are not sufficiently protective of public health?

The World Health Organization (WHO) has acknowledged the condition of electrohypersensitivity, and published a 2006 research agenda for radio-frequency fields (see Addendum to Chapter 12 on the Swedish Government response to persons with Electrosensitivity). The WHO recommends that people reporting sensitivities receive a comprehensive health evaluation. It states: "Some studies suggest that certain physiological responses of EHS individuals tend to be outside the normal range. In particular, hyperactivity in the central nervous system and imbalance in the autonomic nervous system need to be followed up in clinical investigations and the results for the individuals taken as input for possible treatment." Studies of individuals with sensitivities ought to consider sufficient acclimatization of subjects as recommended for chemical sensitivities, as well as recognition of individuals' wavelength-specific sensitivities. Reduction of electromagnetic radiation may ameliorate symptoms in people with chronic fatigue.

Off-gassing of electrical equipment may also contribute to sensitivities. Different sorts of technology (e.g. various medical equipment, analogue or digital telephones; flat screen monitors and laptop computers or larger older monitors) may vary significantly in strength, frequency and pattern of electromagnetic fields. One challenging question for science is to find out if, for instance, 50- or 60-Hz ELF pure sine wave, square waves or sawtooth waveform, ELF-dirty (e.g. radiofrequencies on power lines), ELF-modulated radiofrequency fields, continuous wave radiofrequency radiation and particularly pulsed radiofrequency signals are more or less bioactive, e.g. as neurotoxic and/or carcinogenic environmental exposure parameters. (see Chapter 8 on Disruption by Modulation).

VIII. Conclusions

- Both human and animal studies report large immunological changes with exposure to environmental levels of electromagnetic fields (EMFs). Some of these exposure levels are equivalent to those of e.g. wireless technologies in daily life.

- Measurable physiological changes (mast cells increases, for example) that are bedrock indicators of allergic response and inflammatory conditions are stimulated by EMF exposures.

- Chronic exposure to such factors that increase allergic and inflammatory responses on a continuing basis may be harmful to health.

- It is possible that chronic provocation by exposure to EMF can lead to immune dysfunction, chronic allergic responses, inflammatory responses and ill health if they occur on a continuing basis over time. This is an important area for future research.

- Specific findings from studies on exposures to various types of modern equipment and/or EMFs report over-reaction of the immune system; morphological alterations of immune cells; profound increases in mast cells in the upper skin layers, increased degranulation of mast cells and larger size of mast cells in electrohypersensitive individuals; presence of biological markers for inflammation that are sensitive to EMF exposure at non-thermal levels; changes in lymphocyte viability; decreased count of NK cells; decreased count of T lymphocytes; negative effects on pregnancy (uteroplacental circulatory disturbances and placental dysfunction with possible risks to pregnancy); suppressed or impaired immune function; and inflammatory responses which can ultimately result in cellular, tissue and organ damage.

- Electrical hypersensitivity is reported by individuals in the United States, Sweden, Switzerland, Germany. Denmark and many other countries of the world. Estimates range from 3% to perhaps 10% of populations, and appears to be a growing condition of ill-health leading to lost work and productivity.

- The WHO and IEEE literature surveys do not include all of the relevant papers cited here, leading to the conclusion that evidence has been ignored in the current WHO ELF Health Criteria Monograph; and the proposed new IEEE C95.1 RF public exposure limits (April 2006).

- The current international public safety limits for EMFs do not appear to be sufficiently protective of public health at all, based on the studies of immune function. New, biologically-based public standards are warranted that take into account low-intensity effects on immune function and health that are reported in the scientific

IX. Acknowledgements

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Appendix 8-A Some legal aspects of the functional impairment electrohypersensitivity in Sweden

In Sweden, electrohypersensitivity (EHS) is an officially fully recognized functional impairment (i.e., it is not regarded as a disease). Survey studies show that somewhere between 230,000 - 290,000 Swedish men and women, out of a population of 9,000,000 people, report a variety of symtoms when being in contact with electromagnetic field (EMF)-sources.

The electrohypersensitive persons have their own handicap organisation; The Swedish Association for the ElectroSensitive; http://www.feb.se (the website has an English version). This organisation is included in the Swedish Disability Federation (Handikappförbundens SamarbetsOrgan; HSO). HSO is the unison voice of the Swedish disability associations towards the government, the parliament and national authorities and is a cooperative body that today consists of 43 national disability organisations (where The Swedish Association for the ElectroSensitive is 1 of these 43 organisations) with all together about 500,000 individual members. You can read more on http://www.hso.se (the site has an English short version). The Swedish Association for the ElectroSensitive gets a governmental subsidy as a handicap organization according to SFS 2000:7 §2 (SFS = The Swedish Governmental Statute-Book). EHS persons' right to get disablement allowances has been settled in The Swedish Supreme Administrative Court, i.a. in the judgement "dom 2003-01-29, mål nr. 6684-2001".

Swedish municipalities, of course, have to follow the UN 22 Standard Rules on the equalization of opportunities for persons with disabilities ("Standardregler för att tillförsäkra människor med funktionsnedsättning delaktighet och jämlikhet"; about the UN 22 Standard Rules, see website: http://www.un.org/esa/socdev/enable/dissre00.htm). All persons with disabilities shall, thus, be given the assistance and service they have the right to according to the Swedish Act concerning Support and Service for Persons with Certain Functional Impairments (LSS-lagen) and the Swedish Social Services Act (Socialtjänstlagen). Persons with disabilities, thus, have many different rights and can get different kinds of support. The purpose of those rights and the support is to give every person the chance to live like everyone else. Everyone who lives in the Swedish municipalities should be able to lead a normal life and the municipalities must have correct knowledge and be able to reach the persons who need support and service. Persons with disabilities shall be able to get extra support so that they can live, work, study, or do things they enjoy in their free time. The municipalities are responsible for making sure that everyone gets enough support. Everyone shall show respect and remember that such men and women may need different kinds of support.

In Sweden, impairments are viewed from the point of the environment. No human being is in itself impaired, there are instead shortcomings in the environment that cause the impairment (as the lack of ramps for the person in a wheelchair or rooms electrosanitized for the person with electrohypersensitivity). This environment-related impairment view, furthermore, means that even though one does not have a scientifically-based complete explanation for the impairment electrohypersensitivity, and in contrast to disagreements in the scientific society, the person with electrohypersensitivity shall always be met in a respectful way and with all necessary support with the goal to eliminate the impairment. This implies that the person with electrohypersensitivity shall have the opportunity to live and work in an electrosanitized environment.

This view can fully be motivated in relation to the present national and international handicap laws and regulations, including the UN 22 Standard Rules and the Swedish action plan for persons with impairments (prop. 1999/2000:79 "Den nationella handlingplanen för handikappolitiken - Från patient till medborgare"). Also the Human Rights Act in the EU fully applies.

A person is disabled when the environment contains some sort of impediments. It means that in that moment a man or woman in a wheelchair can not come onto the bus, a train, or into a restaurant, this person has a disability, he or she is disabled. When the bus, the train or the restaurant are adjusted for a wheelchair, the person do not suffer from his disability and are consequently not disabled. An electrohypersensitive person suffers when the environment is not properly adapted according to their personal needs. Strategies to enable a person with this disability to attend common rooms such as libraries, churches and so on, are for instance to switch off the high-frequency fluorescent lamps and instead use ordinary light bulbs. Another example is the possibility to switch off - the whole or parts of - the assistive listening systems (persons with electrohypersensitivity are often very sensitive to assistive listening systems).

In the Stockholm municipality - were I live and work as a scientist with the responsibility to investigate comprehensive issues for persons with electrohypersensitivity - such persons have the possibility to get their home sanitized for EMFs. It means for example that ordinary electricity cables are changed to special cables. Furthermore, the electric stove can be changed to a gas stove and walls, roof and floors can be covered with special wallpaper or paint with a special shelter to stop EMFs from the outside (from neighbours and mobile telephony base stations). Even the windows can be covered with a thin aluminum foil as an efficient measure to restrain EMFs to get into the room/home. If these alterations turn out not to be optimal they have the possibility to rent small cottages in the countryside that the Stockholm municipality owns. These areas have lower levels of irradiation than others. The Stockholm municipality also intend to build a village with houses that are specially designed for persons who are electrohypersensitive. This village will be located in a low-lewel irradiation area. [One of my graduate students, Eva-Rut Lindberg, has in her thesis project studied the "construction of buildings for persons with the impairment electrohypersensitivity". The doctoral thesis will be presented during the Autumn.]

Persons with electrohypersensitivity also have a general (legal) right to be supported by their employer so that they can work despite of this impairment. For instance, they can get special equipment such as computers that are of low-emission type, that high-frequency fluorescent lamps are changed to ordinary light bulbs, no wireless DECT telephones in their rooms, and so on.

Some hospitals in Sweden (e.g. in Umeå, Skellefteå and Karlskoga) also have built special rooms with very low EMFs so that persons who are hypersensitive can get medical care. Another example is the possibility for persons who are electrohypersensitive to get a specially designed car so that the person can transport himself/herself between his/her home and their workplace.

Recently, some politicians in the Stockholm municipality even proposed to the politicians responsible for the subway in the Stockholm City that a part of every trainset should be free from mobile phones; that the commuters have to switch of the phones in these selected parts to enable persons with electrohypersensitivity to travel with the subway (compare this with persons who have an allergy for animal fur whereupon people consequently is prohibited to have animals, such as dogs or cats, in selected parts of the trainset).

In addition, when the impairment electrohypersensitivity is discussed it is also of paramount importance that more general knowledge is needed with the aim to better adapt the society to the specific needs of the persons with this impairment. The Swedish "Miljöbalk" (the Environmental Code) contains an excellent prudence avoidance principle which, of course, most be brought into action also here, together with respect and willingness to listen to the persons with electrohypersensitivity.

Naturally, all initiatives for scientific studies of the impairment electrohypersensitivity must be characterized and marked by this respect and willingness to listen, and the investigations shall have the sole aim to help the persons with this particular impairment. Rule 13 in the UN 22 Standard Rules clearly says that scientific investigations of impairments shall, in an unbiased way - and without any prejudice - focus on cause, occurrence and nature and with the sole and explicit purpose to help and support the person with the impairment.

A unique conference recently was held in Stockholm in May, 2006. The theme for the conference was "The right for persons with the impairment electrohypersensitivity to live in a fully accessible society". The conference was organized by the Stockholm City municipality and the Stockholm County Council and dealt with the most recent measures to make Stockholm fully accessible for persons with the impairment electrohypersensitivity. Among such measures are to offer home equipment adjustments, ban mobile phones from certain underground cars as well as certain public bus seats, and through electrosanitized hospital wards. The conference was documented on film.

SECTION 8

Evidence for Effects on the Immune System Supplement 2012

Immune System and EMF RF

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I. INTRODUCTION

Population exposure to electromagnetic fields (EMF) from mobile phones is continuous and long-term. Unfortunately this is still not taken into account in international standards. Thus it is important to consider immunological studies that relate to chronic and long-term exposure to EMF since the immune system was considered as a critical system in studies conducted in the former USSR. The results of these studies were important for developing standards in the former USSR and the current Russian exposure limits.

Both national and international scientists have studied the immune system as a possible critical system from short exposure to radiofrequency (RF) fields of low intensity (Fiskeko et al. 1999a; Novoselova et al. 1999; Kolomeitcheva et al. 2002; Cleary et al. 1990; Czerska et al.1992; Moszczynski et al. 1999; Stankiewicz et al. 2006; Nasta at al. 2006, Prisco et al. 2008; Johansson 2009; Pinto et al. 2010; Sambucci et al. 2010; Ait-Aissa et al. 2012 and others). These studies were performed under different conditions of EMF exposure as well as different methods and end-points. Analysis of these study results still does not allow criteria for standards development. However, there are only a few studies that are important and were performed in the 1970-1990s by scientists at the Kiev Institute of Public Hygiene headed by Academician Mikhail Shandala (Dronov and Kuritseva 1971; Vinogradov and Dumanski, 1974, 1975; Shandala and Vinogradov, 1982; Vinogradov et al. 1985; Shandala, et al.1983, 1985; Vinogradov and Naumenko, 1986; Vinogradov et al.1987; Vinogradov et al, 1991).

It should be emphasized that these studies were conducted many years ago using methodological recommendations published by the Ukrainian Ministry of Health in 1981 on evaluation of biological actions of microwave radiation of low intensity necessary for development of hygienic regulations (Ukrainian Ministry of Health 1981). Using these recommendations all studies were conducted under the same conditions and so subsequent studies can be considered as a replication of the previous studies that was important for the validity of the final results.

In the first pilot studies conducted in the beginning of the 1970s it was shown that exposure to RF with power density of 15 $\mu W/cm^2$ resulted in disruption of the antigen structure of brain tissue leading to the formation of sensitized lymphocytes and the development of autoimmune reactions.

These studies have been described and translated by Repacholi et al (2012) and part of the translation from this paper has been incorporated here.

Dronov and Kiritseva (1971) exposed 15 rabbits to 50 μW/cm^2 and 5 rabbits to 10 μW/cm^2 UHF (no frequency given) fields for 4h/day for 4 months. The 15 animals exposed to 50 μW/cm^2 were divided into 3 groups of 5 animals each; the 1st group was sensitized (injected with an antigen) during exposure, the 2nd group sensitized before exposure, and the 3rd group sensitized after exposure. The 10 μW/cm^2 group was sensitized during exposure. Immunological changes were assessed using the agglutination reaction, the reaction to indirect hemagglutination, and differential determination of macro- and micro-globulin antibodies with a sedimentation constant of 19S (IgM) and 7S (IgG), respectively. The authors reported that 50 μW/cm^2 caused a decreased antibody response only when exposure occurred prior to or during sensitization and no effect was produced from the 10 μW/cm^2 exposure.

Vinogradov and Dumanski (1974) exposed white rats EMF 2450MHz at 50 μW/cm^2 for 5 h/day for 14 days. The authors reported alterations to the structure and/or expression of tissue antigens using the method of anaphylaxis with desensitization. In this study 25 white rats were included, of which 20 were UHF exposed (PD of 50 μW/cm^2). Sera from these and 5 control animals were investigated for the content of antibodies against normal and exposed animals, using the complement binding reaction in the cold. The reaction was started immediately after exposure and weekly afterwards for one month. The results of theses experiments are shown in Table 1.

Table 1. Complement binding reaction in white rats after UHF exposure (M ± m)
(Vinogradov and Dumansky 1974 modified from Repacholi et al. 2012)

Antigen from brain tissue of	Background		Immediately after radiation		After 1 week		After 2 weeks		After 3 weeks		After 4 weeks	
	No. of positive reactions	Log_{10} antigen titre	No. of positive reactions	Log_{10} antigen titre	No. of positive reactions	Log_{10} antigen titre	No. of positive reactions	Log_{10} antigen titre	No. of positive reactions	Log_{10} antigen titre	No. of positive reactions	Log_{10} antigen titre
Exposed rats	0	0	7	1.60±0.19	17	$2.1±0.11^{*}$	18	$2.46±0.2^{**}$	18	$2.51±0.06^{**}$	5	1.54±0.31
Normal rats	0	0	6	1.50±0.14	18	1.80±0.13	16	1.95±0.06	4	1.45±0.18	0	0

* $p < 0,05$
** $p < 0,01$

The authors concluded RF exposure could induce expression of antigens not normally expressed in brain tissues and/or alter antigen structure of normally expressed antigens.

Therefore these early studies established that exposure to RF at power density (PD) of 50 µW/cm^2 could result in changes in antigenic structure of tissue and blood proteins. These changes were characterized by the appearance of new nonspecific antigenic qualities and partial elimination of normal antigens, i.e. the exposure resulted in changes of antigenic structure of tissues. However, this conclusion required confirmation and further exploration. As a result a few subsequent studies were performed at longer long-term RF exposures.

Vinogradov and Dumanski (1975) reported that exposure to 2450 MHz fields 7h/day for 30 days at 50 µW/cm^2 induced autoantibodies reacting with brain tissue antigens in Guinea pigs, white Wistar rats and rabbits. Autoimmune reactions were identified using the complement binding reaction (CBR) and plaque forming cell techniques that revealed the presence of antigen-specific antibodies and antigen-specific antibody-producing cells, respectively. Moreover, leukocytes from UHF-exposed Guinea pigs showed a reduced serum-mediated phagocyte activity.

To obtain the antigen from exposed brain tissue, brains from donor animals, housed under the same conditions as experimental ones, were sacrificed immediately at the end of the exposure cycle. Blood to conduct the CBR was collected according to the following schedule: background, immediately after exposure, and then after 2, 4, 6, and 8 weeks after exposure. The results are shown in Table 2. The study showed that RF exposure of animals (guinea pigs and rats) at 50 µW/cm^2 resulted in the alteration of protein structure in brain tissues and production of circulating brain antigens.

Sampling time	Guinea pigs			White rats		
	No. of reactions	No. of positive reactions	Log_{10} of antibody titres (M±m)	No. of reactions	No. of positive reactions	Log_{10} of antibody titres (M±m)
Background	24	0	-	20	0	-
Immediately after exposure	24	19	1.95 ± 0.06	20	7	1.60 ± 0.19
2 weeks after exposure	24	20	2.77 ± 0.04	20	18	2.46 ± 0.2
4 weeks after exposure	24	20	2.56 ± 0.05	20	18	2.51 ± 0.06
6 weeks after exposure	24	18	2.05 ± 0.07	20	19	2.10 ± 0.11
8 weeks after exposure	24	13	1.71 ± 0.05	20	5	1.54 ± 0.31

Table 2. Dynamics of titres of antigens against brain in Guinea pigs and white rats after UHF exposure at 50 µW/cm^2 ,Vinogradov and Dumansky 1975 (From Repacholi et al. 2012)

The results shown in Table 2 indicate a time-dependence in the formation of circulating antibodies against the brain. The antibody titre in Guinea pigs increased in time after the exposure and reached a maximum 2 weeks after exposure ($\log_{10}$ of the titre was 2.77 ± 0.04). The authors concluded that chronic exposure to RF at a PD of 50 μW/cm^2 resulted in the formation of brain antigens in the animals. This process was observed using brain tissue from both exposed and non-exposed animals. The highest titres of compliment binding were observed 10-14 days after exposure.

The results of the subsequent study, published in the same paper (Vinogradov and Dumansky 1975), indicated a similar time-dependent trend suggesting that the action was consistent. The authors investigated the cellular auto-immune reaction by determining the number of spot forming cells, synthesising antibodies against its own erythrocytes in the blood. The study was conducted on Guinea pigs and white rats that were exposed for one month to UHF fields at a PD of 50 μW/cm^2. The Jerne reaction in blood was performed before exposure, immediately after the end of exposure, and then after 2 and 4 weeks. Results of the study are shown in Table 3.

Animal species	No. of animals	Background	Immediately after exposure	2 weeks after exposure	4 weeks after exposure
Guinea pigs P-value	10	2.1 ± 0.21	2.8 ± 0.4 > 0.05	14.7 ± 1.1 < 0.001	9.01 ± 0.6 < 0.001
White rats P-value	7	1.5 ± 0.15	1.57 ± 0.20 > 0.05	10.4 ± 1.0 < 0.001	6.7 ± 0.8 < 0.001

Table 3. Percentage of spot forming cells from Guinea pigs and white rats after UHF monthly exposure at a PD of 50 μW/cm^2 (M±m),
Vinogradov and Dumansky 1975 (Modified from Repacholi et al. 2012)

As seen from Table 3, a statistically significant increase in the percentage of spot forming cells was observed during the second week after exposure and was quite stable. Four weeks after the exposure the % still remained high.

Subsequently the same authors (Vinogradov and Dumansky, 1975) performed a study to investigate adverse properties of blood serum after UHF exposure based on the determination of changes in the phagocytic capacity of the cells. Fifteen Guinea pigs were included in the study, which were exposed to UHF at a PD of 50 μW/cm^2 for 1 month. Phagocytosis was determined three times – before exposure and 2 and 4 weeks after the exposure. Table 4 shows the results of phagocytosis in three stages of the study. These data indicate that serum from the exposed animals has a pronounced suppressive effect both on phagocyte number and the phagocyte index. This effect was pronounced in blood serum collected 2 weeks after exposure and remained for another 2 weeks.

Guinea pig serum before exposure		Guinea pig serum 2 weeks after exposure		Guinea pig serum 4 weeks after exposure	
Phagocyte no.	Phagocyte index	Phagocyte no.	Phagocyte index	Phagocyte no.	Phagocyte index
63.4 ± 3.2	6.28 ± 0.5	29.6 ± 2.4 $P < 0.001^{*}$	3.61 ± 0.56 $P < 0.01^{**}$	22.9 ± 3.0 $P < 0.001^{*}$	4.10 ± 0.6 $P < 0.05^{**}$

[*] compared to the phagocyte number in Guinea pig before exposure
[**] compared to the phagocyte index in Guinea pig before exposure

Table 4. Suppression of the phagocyte reaction under the influence of sera from exposed animals, Vinogradov and Dumansky 1975(From Repacholi et al. 2012)

Considering the results of these three studies it can be concluded that long-term RF exposure at low intensity (50 µW/cm^2) results in auto-allergic reactions.

Shandala et al. (1983) exposed CBA mice and Wistar rats to 2375 MHz (7 h/day). When mice were exposed to 0.1 or 10 mW/cm^2 it increased spontaneous and mitogen-stimulated (PHA) cell proliferation, which persisted for 30 days after the last exposure. When rats were exposed for 3 months to 1 or 5 µW/cm^2 or for 1 month at 10, 50, 500 µW/cm^2, there was a decrease in proliferative response to PHA, still evident 3 months post exposure. No effects were observed with 10 and 50 µW/cm^2 in rats. The authors concluded that RF exposure induced important changes in T-cell immunity.

Vinogradov et al. (1985) exposed white Wistar rats for 30 days to 10, 50, 500 µW/cm^2 (2375 MHz) and a sham-exposed group used as controls. Induction of autoantibodies toward brain tissue antigens (brain extracts) was evaluated with the complement binding/fixation assay and pathological effects assessed by injecting auto-antibody-containing sera into pregnant animals. Electrophoresis patterns of sera immunoglobulin were also evaluated. Exposure to 50 and 500 µW/cm^2 induced autoantibodies to brain tissue antigens as revealed by indirect degranulation of basophiles and complement fixation assays. No effects were induced from exposure to 10 µW/cm^2. Exposure to 50 and 500 µW/cm^2 also decreased cell proliferation (blast formation). Sera from exposed (or sham-exposed) rats were injected into pregnant rats to verify whether the presence of the autoantibodies was pathological. Sera from rats exposed to 500 µW/cm^2 increased post-implantation loss and decreased the number, body weight and length of the newborns. Analyses of soft tissues from the fetuses revealed the presence of hemorrhage in subcutaneous tissues, peritoneal cavity, liver and brain. The authors also reported that exposure to 500 µW/cm^2 (but not 10 µW/cm^2 or 50 µW/cm^2) led to alterations in immunoglobulin electrophoresis, with the appearance of a new peak similar to that of class A antibodies, and concluded that it caused strong changes in physico-chemical and immunological properties of serum humoral factors. The authors concluded that such changes might render proteins naturally produced in the body as immunologically “foreign” and stimulate auto-immune responses.

To repeat the results of Shandala et al. (1985) and Vinogradov and Naumenko (1986) exposed Wistar rats to 2375 MHz fields at 50 or 500 µW/cm² for 30 days for 7 h/day and confirmed that exposure to 500 µW/cm² induced anti-brain antibodies using complement binding and basophiles degranulation assays, and increased plaque-forming cells, suggesting RF exposure altered brain tissues rendering them immunogenic. When rats were injected with extracts from animals exposed to 500 µW/cm² the authors also reported an increased number of reticulo-endothelial and plasma cells in bone marrow and spleen and a decreased number of small lymphocytes in bone marrow.

Vinogradov et al. (1991) exposed female Fisher rats to 2375 MHz (500 µW/cm², 7 h/day for 15 days). Exposure effects were assessed by injecting lymph node cells from exposed or sham-exposed animals into normal recipient rats. This was to determine if it was possible to transfer the “conditions of autoimmunity caused by the exposure” into recipient animals. Analyses were then performed on both donor and recipient rats and, consistent with previous reports, the authors found exposure reduced mitogen-stimulated cell proliferation (PHA and Con A) and induced auto-antibodies toward brain tissue antigens as shown by basophiles degranulation and plaque forming cell assays. Moreover, cells injected from exposed animals (but not from sham-exposed rats) “led to analogous conditions” in normal recipient rats.

Shandala and Vinogradov (1982) exposed 11 pregnant white Wistar rats to UHF (500 µW/cm^2, 7 h/day for 30 days) and reported an increased response to fetal liver antigens in terms of both frequency of antibody-producing lymphocytes in blood and auto-antibodies in serum, compared to 11 unexposed controls. Lymphocytes from exposed pregnant rats also showed a reduced mitogen-stimulated cell proliferation compared with controls. When sera were injected into pregnant rats (10 exposed and 10 controls) “to evaluate the pathological meaning of the auto-antibodies”, sera from exposed rats increased embryo lethality during pregnancy and higher offspring mortality at around 1 month of age.

Shandala et al. (1985) exposed female Wistar rats to UHF fields (2375 MHz) at 50 and 500 µW/cm^2 for 7 h/day for 30 days. They investigated induction of autoantibodies and found these exposures induced the formation of autoantibodies to brain tissue extract using the basophiles degranulation technique. The authors then investigated the immunogenicity of brain extracts from exposed animals by injecting these extracts into normal animals. Their hypothesis was that normal tissue should not induce antibodies to brain tissue since recipient animals should recognize them as their own tissues. If exposure to UHF induced alterations in antigen expression and/or structure, the tissue extract should become immunogenic and therefore able to raise an antibody response. The authors reported that brain tissue extracts from animals exposed to 50 and 500 µW/cm^2 induced antibodies in injected animals, but basophiles degranulation was seen only in animals injected with extracts from animals exposed to 500 µW/cm^2. To assess the pathological significance of the autoantibodies they injected sera from animals exposed to 500 µW/cm^2 into pregnant rats and this increased post-implantation loss. No effects were induced by the injection of sera from animals exposed to 50 µW/cm^2. The authors concluded that only exposure to 500 µW/cm^2 was capable of inducing anti-brain antibodies, leading to an adverse effect.

When Vinogradov et al. (1987) reviewed the results of these immunological studies they concluded that exposure to UHF at a power density of 500 µW/cm^2 irreversibly damages organisms while 50 µW/cm^2 induces some effects often non pathogenic, and 10 µW/cm^2 does not affect any immunological parameters. This early assessment seems to have been given much credence by all subsequent standards committees.

When the public health standards committees analyzed all studies they agreed with Vinogradov et al. (1987):

- 100-500 µW/cm^2 chronic daily exposure can induce persisting pathological biological reactions (based on the immunology studies above), the most striking effect being offspring death after injection of foreign serum.
- ~ 50 µW/cm^2 is the threshold exposure for unfavorable biological effects (based on the immunology studies above). These effects were not pathological since the organism could compensate for the exposure but continual compensation could lead to long-term adverse effects and thus should be protected against.
- ≤10-20 µW/cm^2 chronic exposure does not induce any noticeable biological changes in small laboratory animals.

Therefore, specialists from the Kiev Institute in 1970-1980s showed that there was a clear dose-dependence in biological effects of RF on the immune system. Chronic RF exposure at 500 µW/cm^2 in the frequency range 1750-2750 MHz resulted in significant changes in the immune status of immunocompetent globulin fractions, and changes in antigenic structure of tissue and blood proteins resulted in the development of autoimmune processes. Chronic exposure at 1-20 µW/cm^2 did not result in changes to immunological status. These results, as well as studies of other systems of the animal chronically exposed to RF fields at the same PDs were used for establishing the first standards in the former USSR.

Russian-French study performed under WHO EMF project (2006-2009)

Considering the importance of the results obtained in 1970-1980s (described above) for harmonization of standards (performed in a special program on development of a scientific basis for setting standards for RF EMF) the International Advisory Committee of the World Health Organization's (WHO) Program "EMF and health" included in 2006 research agenda to perform studies to attempt to replicate the results of the earlier immunological studies.

With the purpose to replicate and confirm the results of the earlier Soviet studies we selected two major immunological and teratological studies described above; these were Vinogradov and Dumansky 1974 and Shandala and Vinogradov 1982.

In our replication study the original scientific methods were used, but a modern exposure system, dosimetric and biological methods were used. The study was conducted in a blind manner; in addition to the CBR, the ELISA test was used to evaluate immunological responses induced by RF exposure.

Preparatory work for the replication study began in 2006: a program and detailed protocol of the study were developed and were subsequently discussed and agreed with WHO and approved by an independent International Advisory Committee (IAC), who included scientists from Germany (J. Bushmann), Italy (C. Pioli) and USA (R. Sypnewski). The Committee was chaired by the head of WHO EMF project Dr. Mike Repacholi.

With agreement with WHO, the former SRC Institute of Biophysics (now the Federal Medical Biophysical Centre of FMBA, Moscow, Russia) was chosen to implement the study. Animal exposure and dosimetric evaluations were jointly performed by specialists from the Centre for Electromagnetic Safety (Moscow, Russia) and the IMS laboratory (University of Bordeaux, France). The RF exposure conditions were jointly agreed by the scientific group and the IAC. The exposure geometry resulted in relatively uniform exposure of animals in the study as confirmed by dosimetric evaluations.

Scientists in the key specialties were invited to perform the replication study. During the quarantine period (14 days) and exposure period (30 days) the animals were handled in a blind manner by scientists from the radiobiological laboratory of the Institute of Biophysics (supervised by Prof. N.G. Darenskaya).

The replication study began in October 2006. The International Advisory Committee monitored all steps of the study, including the final results and conclusions. The final scientific report and conclusions of the replication study were reviewed by IAC. The main results of the study were published in English in "Bioelectromagnetics" journal (Grigoriev et al. 2010a) and as a series of papers in Russian in the "Radiation Biology. Radioecology" journal (Grigoriev et al. 2010, Lyaginskaya et al. 2010). English translation of these papers was published in "Biophysics" journal (Grigoriev et al. 2010b-e, Lyaginskaya et al. 2010).

The following section briefly describes this replication study (Grigoriev et al 2010a-e).

The study of immunological and reproductive effects of long-term low-level microwave exposure was conducted on Wistar (WI) rats in a blind manner. There were three groups of rats, each consisting of 16 males: (1) the RF-exposed group included rats that were exposed to low-intensity RF in an anechoic chamber, (2) the sham-exposed group included rats that were treated in the same way as (1) but were not RF-exposed, and (3) the cage control group included rats kept in the animal room. Rats from each group were donors of blood serum and tissues on the 7th and 14th day after termination of the exposure. The immunology study was performed on blood serum and brain and liver extracts taken at both time points. In the study on pre- and early postnatal development of offspring, blood taken on the 14th day after the exposure from Sham-exposed and RF-exposed rats was injected into pregnant rats on the 10th day of pregnancy. For the latter study mature rats (90 females and 30 males) were used.

The exposure system and conditions were made as similar as possible to those in the original studies (Vinogradov and Dumansky, 1974,1975; Shandala and Vinogradov, 1982; Vinogradov and Naumenko, 1986). Rats were exposed in the far field to an elliptically polarized 2450 MHz continuous wave RF field from above the ring at an incident power density of 5 W/m^2 at the cage location for 7 h/day, 5 days/week for a total of 30 days of exposure. Actual and Sham RF exposure was carried out in two shielded anechoic chambers. The Sham and RF-exposed animals were placed in special cages arranged in a ring in each chamber (Fig. 1). The cages (Atelier Deco Volume, Limoges, France) were made of dielectric materials, Plexiglas and PVC, with holes for ventilation. Each ring consisted of 16 cages with one rat per cage. Rats were free to move and cages were covered with transparent lids.

RF was generated by a diathermy unit, SMV-150-1 ''Luch-11'' magnetron (Electronic Medical Apparatuses (EMA), Moscow, Russia), with a standard helical antenna having an external diameter of 90 mm. The generator produced continuous RF at 2450 ± 50 MHz and was connected to the antenna using a feeder about 8.5 m long, made of RK50-11-21 coaxial cable (Kazenergokabel, Pavlodar, Kazakhstan) with Teflon insulation. The antenna was fixed 2.35 m above the floor in chamber 2, and was mounted on a bracket made of plastic and wood (Fig. 1). The output of the ''Luch-11'' was set to 71.0 ± 7.3 W antenna input power.

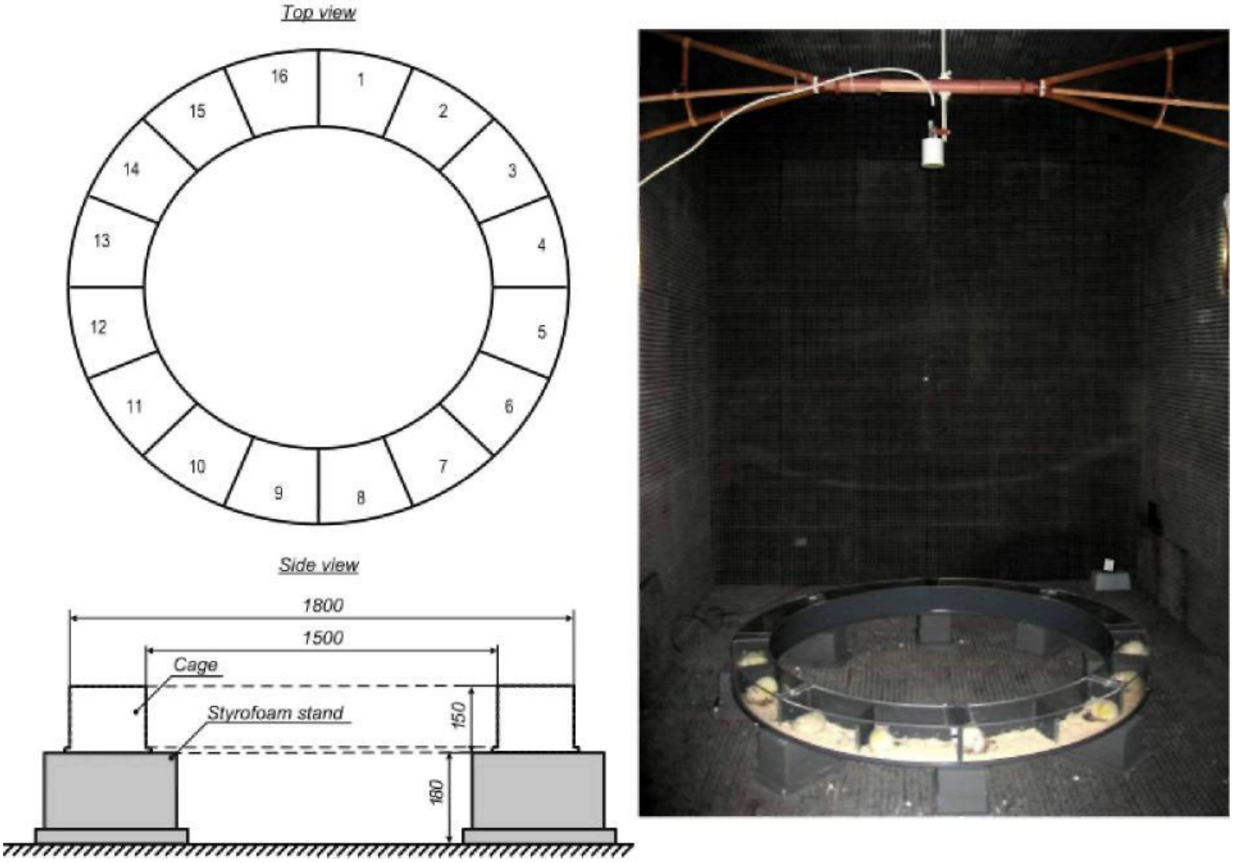

Fig. 1. General scheme of the RF exposure setup, illustrating the ring containing the cages for the animals (sketch) and the fixed antenna above the ring (from Grigoriev et al. 2010a)

Measurements of equivalent plane wave power density were made using a Narda EMR-20 broadband meter (Pfullingen, Germany), connected to a personal computer through a fiber-optic link. A detailed description of the exposure conditions and dosimetric measurements is provided in Grigoriev et al. 2010a. Dosimetric calculations were performed by Dr. Philippe Leveque, the contracted dosimetrist for our study. They showed that the whole-body SAR evaluated for the exposure conditions was 0.16 ± 0.04 W/kg. The averaged SAR in the brain was about 0.16 W/kg. A maximum peak SAR value of 9.9 W/kg was calculated in the tail skin; maximum peak SAR value for the brain was 1.0 W/kg. After termination of the exposure, rat tissues were sampled for the two studies (immunological and teratological).

Study of the effects on the immune system

The immunological study was performed using the Complement Fixation Test (or Complement Binding Reaction) at low temperature (Shubik, 1987) and the modern ELISA test.

The Complement Fixation Test (CFT) was used to evaluate the ability of antibodies (mainly IgM subclass) in blood to react with antigens in brain and liver extracts (Sinaya and Birger, 1949; Birger, 1982).

The CFT was implemented in the same manner as the original Soviet studies. Blood serum, brain and liver were taken from five rats from each group on the 7th day after 30-day RF exposure and from 11 rats from each group on the 14th day after 30-day RF exposure.

The methods of blood sampling and preparation of tissue homogenates from brain and liver were the same as in the original Soviet studies (Vinogradov and Dumansky, 1974, 1975; Vinogradov and Naumenko, 1986). They are described in detail in Grigoriev et al. 2010a.

The reaction of complement fixation was conducted on six different blood serum dilutions in physiological saline solution (1:5, 1:10, 1:20, 1:40, 1:80 and 1:160) with respective brain/liver homogenates, and the outcome of the reaction was judged by a group of three experts for visual assessment of the amount of precipitate and liquid color.

The ELISA test was used to evaluate immunological responses induced by RF exposure via analysis of the level of antibodies reacting with selected antigens (Semballa et al., 2004; Nasta et al., 2006; Mangas et al., 2008). This test was not used in the original Soviet studies. ELISA was performed using the blood serum samples collected for the CFT on days 7 and 14 after the exposure. Circulating antibodies (IgA, M and G isotypes) were evaluated for 16 antigens, selected by our French collaborators based on the results of the earlier Soviet studies suggesting autoimmune and degenerative processes (Grigoriev et al 2010a).

The results of our CFT showed that there were no statistically significant differences in the levels of antibodies against brain (or liver) antigens between the three groups on day 7 after termination of RF exposure (Grigoriev et al 2010a). On day 14 after RF exposure, an increase in the median serum dilution was seen in the reaction with brain homogenates in the three studied groups compared to the median levels registered on day 7. Only in the control group the increase was not statistically significant; in the Sham-exposed group the median serum dilution increased from 1:5 to 1:10, and in the RF-exposed group the increase was more pronounced, from 1:5 to 1:20. The levels of antibodies against liver antigens did not change significantly. On day 14 after termination of the exposure, the difference in levels of antibodies against brain antigens between RF- and Sham-exposed groups became statistically significant ($P < 0.01$). However, our CFT results showed that the difference between the Sham-exposed and control groups was almost significant, which could be explained by stress and other factors. The appearance of antibodies against liver antigens was smaller than against brain antigens (Grigoriev et al 2010a). The results of our CFT are shown in Fig. 2 in units used in the original studies.

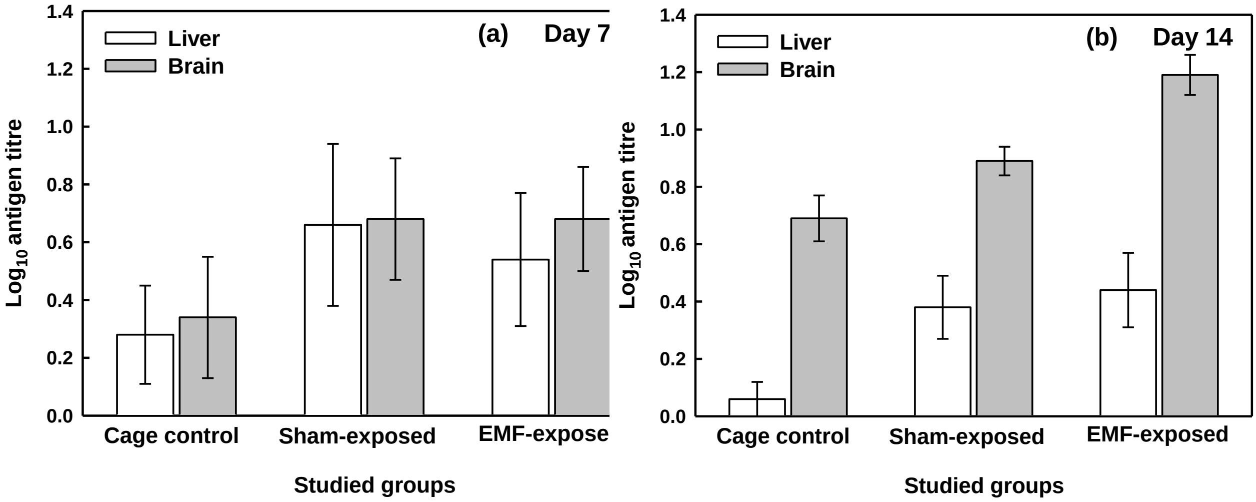

Fig.2. Average $\log_{10}$ antigen titre in the three groups of rats on day 7 (a) and day 14 (b) after the termination of the exposure shown for liver (white boxes) and brain (grey boxes) antigens. Vertical bars represent standard errors. The results are shown in units used in the original studies.

In our opinion, a notable increase in the level of antibodies against brain antigens seen in the Sham- and RF-exposed groups of rats on day 14 after termination of the 30-day RF exposure could be explained by long-term hypokinesia (reduced movement during the whole experiment) and stress reactions of the animals. It is known that hypokinesia in space (Ivanov and Shvets, 1978) or in laboratory animals (Portugalov et al., 1976) results in an increase in autoantibodies in blood serum available for complement fixation. However, on the 14th day after the 30-day exposure, the increase in antibodies against brain antigens in the RF-exposed group was statistically different from the Sham-exposed group, even noting their state of hypokinesia. Comparison of our results with the results of earlier Soviet studies showed that the formation of antibodies against brain antigens was less pronounced in our study but the general trend was similar. It should be noted that the earlier studies evaluated characteristics of immunity using different parameters that allowed a more reliable estimate of the expression of autoimmune processes due to chronic non-thermal RF exposure. However, assessment and analysis of these parameters was not included in our replication study.

Results of the evaluation of circulating antibodies directed against 16 antigens using the ELISA test showed that there was an increased number of compounds resulting from interaction of amino acids with NO or its derivatives (NO_2-tyrosine, NO-arginine, NO-cysteine+NO-bovine serum albumin, NO-methionine+NO-asparagine+NO-histidine, NO-tryptophan+NO-tyrosin), as well as fatty acids with short chains (C6-C8-C10-C12; C6-C8-C10-C12; PAL/MYR/OLE) in blood serum from RF-exposed rats. Fig. 3 shows content of antibodies (IgM and IgG subclasses) to products of interaction of amino acids with nitric oxide NO or its derivatives (NO_2-tyrosine, NO-arginine, NO-cysteine+NO-bovine serum albumin, NO-methionine+NO-asparagine+NO-histidine, NO-tryptophan+NO-tyrosin) on days 7 (a) and 14 (b) after the termination of the exposure. Levels of antibodies of IgA subclass were below detection limit.

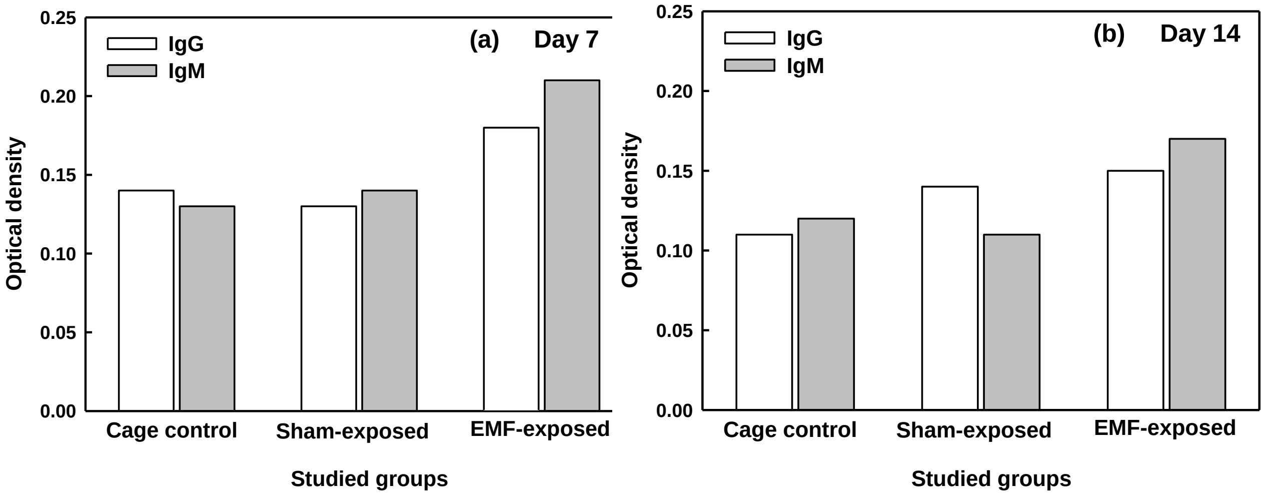

Fig. 3. Content of antibodies (IgM and IgG subclasses) to products of interaction of amino acids with nitric oxide (NO) or its derivatives in blood of rats from the three studied groups on days 7 (a) and 14 (b) after the termination of the exposure (median optical densities)

Antibodies to AZE (product of oxidation of fatty acids) were determined only in the IgM fraction on day 7 after the exposure, and median ODs were equal to 0.31, 0.20 and 0.21 in RF-exposed, Sham-exposed and control groups, respectively. The difference between the RF- and Sham-exposed groups was statistically significant ($P < 0.05$). Enhanced production of these compounds that activate the peroxidation of lipids, the decreased production of antioxidants and the failure of DNA and protein-repair processes result in cellular oxidative stress. In our study, development of oxidative stress was weak and short-term. The maximum content of antigen-specific bound antibodies was seen on day 7 after termination of the RF exposure and subsequently decreased on day 14 (Grigoriev et al 2010a). The response was weak to ANT/ XANT/3OH ANT and was absent for the remaining antigens (3OH Kyn, CAT, MDA+4HNE, Pi, QUINA). As a rule, antibodies to conjugated antigens were seen for IgM, rarely seen for IgG, and were completely absent for IgA. The levels of antibodies were higher on day 7 after exposure compared to those on day 14 after exposure and the differences were not statistically significant between the control and Sham-exposed groups. However, in the RF-exposed group the difference in the levels of antibodies on days 7 and 14 was statistically significant (Grigoriev et al 2010a).

On the whole, our CFT study showed the same tendency of RF exposure to influence the formation of antibodies to brain tissue homogenates as the results of the earlier Soviet-era studies. However, our study showed that quantitative interpretation of the CFT outcomes was rather complex and could be influenced by assumptions accepted in the study. The ELISA test supported our views on the occurrence of intracellular oxidative stress reactions from RF exposure, showing possible development of pathological processes if an unfavorable influence remained.

Study of the effects on pre- and postnatal development of offspring

The animal model in the teratology study on investigation of the exposed blood serum on reproductive endpoints was similar to the one used in an earlier study conducted by Shandala and Vinogradov (1982). Three groups of rats were in this study. The first group (group 1) comprised 17 sperm-positive female rats that served as controls. The second group (group 2) consisted of 21 female rats to which 1ml of blood serum from Sham-exposed rats, taken on day 14 after the exposure, was injected IP on day 10 p.c. The third group (group 3) included 21 female rats to which 1 ml of blood serum from RF-exposed rats, taken on day 14 after the exposure, was injected IP on day 10 p.c.

In utero development and newborns were studied using the following scheme (Grigoriev et al 2010a). On day 15 of pregnancy, 5–6 pregnant female rats from each group were sacrificed to evaluate embryo mortality. Also, the number of implants, corpora lutea of pregnancy, live embryos, resorbed embryos, as well as the mass of the embryos and placentas were recorded in each group of rats. Embryo development and placental formation was assessed by weight. On day 20 of pregnancy, four female rats from groups 2 and 3 were sacrificed to evaluate total in utero mortality and the fertility index; the number of implants and live embryos were also recorded for these rats. In each group, 11–12 pregnant female rats were kept alive until delivery to study offspring development and survival. At delivery, the number of newborns in a litter, body mass of newborns, number of stillborns and apparent birth defects were registered. Study on the effects on postnatal development of the offspring. Offspring development was studied for the first 30 postnatal days using generally accepted integral and specific parameters. Changes in body mass were determined over the first postnatal month by weekly measurements. The specific parameters were appearance of hair cover, detachment of auricles, opening of eyes, eruption of incisors and onset of independent eating.

A response to injection of blood serum was observed in one rat from the Sham-exposed group and three rats from RF-exposed group. These rats were sluggish, slow-moving, refused food and water, and lay rolled up in a ball most of the time. Such response continued for up to 1 h. Three of the four pregnant rats later delivered normal offspring and one rat from the RF-exposed group had all embryos resorbed.

On day 15 of pregnancy, that is, 5 days after injection of blood serum, the number of live embryos per animal did not differ significantly among the studied groups and was equal to 7.5 ± 0.4, 8.3 ±0.2 and 7.4 ±0.4 in groups 1, 2 and 3, respectively. The average mass of embryos of rats from groups 2 and 3 was similar (190.4 ±5.4 and 185.4 ± 4.7 mg, respectively) and was higher than in the control group (151.1 ± 1.6 mg). The ratios of placenta-to-embryo mass (so-called ‘‘placental coefficient’’) were 1.14 ±0.16, 0.96 ±0.03 and 0.95 ± 0.04 in groups 1, 2 and 3, respectively, and did not differ significantly between each other.

Data on embryo mortality evaluated on day 15 of pregnancy showed that embryo mortality was higher in rats from group 3; however, this was not significantly different compared to the other groups.

On day 20 of pregnancy, that is, 10 days after injection of blood serum, the number of live foetuses per animal did not differ significantly between groups 2 and 3 and was equal to 8.3 ± 0.7 and 7.5 ± 0.8, respectively. The average foetal mass in rats also did not differ significantly between these groups and was equal to 3.8 ±0.1 and 3.7±0.1g, respectively. In utero foetal mortality on day 20 of pregnancy increased compared to that on day 15, and did not differ significantly between the rats from groups 2 and 3, being 19.5 ± 6.3% and 23.1 ± 6.8%, respectively.

All rats from groups 1 and 2 delivered offspring on day 22 of pregnancy; in group 3, two rats delivered offspring on day 22 of pregnancy and another two on day 23. Of the total number of pregnant rats left for delivery, offspring were delivered in 100% of rats in the control group (11 rats from 11 animals); 90% of rats from group 2 (9 rats from 10 animals) and 33.3% of rats from group 3 (4 rats from 12 animals). From the group of rats injected with blood serum from the Sham-exposed animals (group 2) two rats that did not deliver offspring were sacrificed, one was found not to be pregnant, and another had all embryos resorbed. Eight rats from the group injected with blood serum from RF-exposed animals (group 3) that did not deliver offspring were also sacrificed and all were found to have their embryos resorbed. Because the body mass of rats was not measured during pregnancy, it was not known when the resorption of embryos occurred.

Total *in utero* foetal mortality was evaluated using the data on foetal mortality on days 15 and 20 of pregnancy and foetal resorption in rats that were pregnant but did not deliver offspring. Fig.4 shows that total in utero mortality among rats from group 3 was significantly higher compared to rats from groups 1 and 2 (55.6 ±4.0%, 4.3 ±3.0% and 11.7 ±3.3%, respectively).

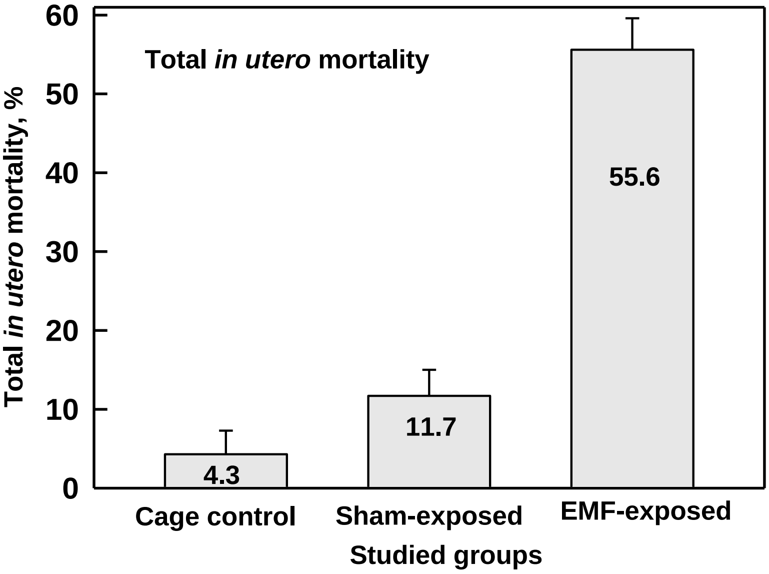

Fig. 4. Total *in utero* mortality in the three groups of rats

The influence on prenatal development was assessed from the number of live foetuses on day 20 of pregnancy and the number of live newborns at delivery. It was shown in our study that in rats from group 3, the number of live foetuses and newborns per pregnant rat (3.8 ± 1.1) was significantly lower than in groups 1 and 2 (8.1 ± 1.1 and 8.7 ±0.8, respectively). However, the number of live foetuses and newborns in rats that had live offspring did not differ significantly between the groups and was equal to 8.1 ± 1.1, 10.2 ±0.9 and 8.7 ±1.3 in groups 1, 2 and 3, respectively (Grigoriev et al 2010a).

High postnatal mortality was observed during the first 30 days of life in our study of offspring mortality and development in the control group (34%). This result does not correspond to the normal outcomes for these rats and our data for postnatal period cannot be used in the analysis.

High *in utero* mortality in rats injected with blood serum from RF-exposed animals (55.6 ± 4.0%) than in female rats injected with serum from Sham-exposed animals (11.7 ± 3.3%) shown in our study suggests a more pronounced embryotoxic effect from RF-exposed serum compared to Sham-exposed serum. The *in utero* mortality in our study was higher than in the study of Shandala and Vinogradov (1982) in all groups of rats. However, we cannot guarantee that the effects depend only on the influence of RF exposure since there was high variability in the following parameters: offspring mortality, mass of embryos, placental coefficient and unusually high mortality in offspring at later ages.

In our opinion, Shandala and Vinogradov (1982) chose a rather complex model that can be subject to variable results and is not an appropriate model for assessing the impact on human health from RF exposure. There are stress responses in the rats, participation of a number of very complex functional systems, and pregnancy itself changes the functional condition of all rat systems. These could all contribute to the wide data scatter seen in our results. It should be noted that our experiment was carried out 25 years after the original study. Unfortunately, a lot of information required to replicate this study was lacking in the original publications, making comparisons with our results more difficult. Because of these problems, we considered the experiment on pre- and early postnatal development of offspring as a pilot study that argues for the necessity of carrying out a larger and more powerful study.

The main conclusions from our study were as follows (Grigoriev et al. 2010a):

- The results of our immunology study using the CFT and ELISA tests partly confirmed the results of the Soviet research groups on the possible induction of autoimmune responses (formation of antibodies to brain tissues) and stress reactions from RF exposure (30-day exposure for7 h/day for 5 days/week at a power density of 5 W/m^2, i.e., long-term non-thermal RF exposure).
- The results of our study on prenatal development of offspring suggested possible adverse effects of the blood serum from exposed rats (30-day exposure for 7 h/day for 5 days/week at a power density of 5 W/m^2) on pregnancy and embryo–foetal development in rats, in agreement with the earlier results of Shandala and Vinogradov (1982), although the model used by Shandala and Vinogradov (1982), which was intentionally replicated here, is not considered an appropriate one for assessing human health effects from RF exposure.

Analysis of the results of our study on RF effects on immune system allowed conclusion that data used in 1976 for development of RF standards in the USSR that are still in action in Russia were reasonable.

In an analogous study performed by our French colleagues using a similar protocol (except that CFT reaction was not implemented) (University of Bordeaux, IMS laboratory) no changes in immune status of animals were registered (Poulletier et al. 2009). However, in our opinion there were a few reasons that could influence the final results of this study. First of all, differences in the status of the experimental animals in these two studies. For example, the average body mass of rats at the end of our study was 275 g, and 400 g in the French study. More detailed discussion of these and other differences between the studies was provided in our comment (Grigoriev 2011).

Analogous results were obtained by our Ukrainian colleagues in a replication study (Tomashevskaya et al 2004). Unfortunately, these results were published as a brief summary in Ukrainian language. This study was conducted in the following conditions: chronic exposure of white outbred rats at 450 MHz for 2 h/day for 4 months. There were three experimental groups of rats exposed at different PDs: 250, 500 and 1000 mW/cm^2 and a sham-exposed group.

II. CONCLUSION

Available data allow the conclusion that the immune system is a critical system for evaluation of the effect of RF at low intensity and should be taken into consideration for development of standards.

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 9

Evidence for Effects on Neurology and Behavior

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I. Introduction

This chapter is a brief review of recent studies on the effects of radiofrequency radiation (RFR) on neuronal functions and their implication on learning and memory in animal studies, effects on electrical activity of the brain and relation to cognitive functions, and finally a section on the effects of cell phone radiation on the auditory system. There is also a set of studies reporting subjective experience in humans exposed to RFR. This includes reports of fatigue, headache, dizziness, and sleep disturbance, etc.

The close proximity of a cellular telephone antenna to the user's head leads to the deposition of a relatively large amount of radiofrequency energy in the head. The relatively fixed position of the antenna to the head causes a repeated irradiation of a more or less fixed amount of body tissue, including the brain at a relatively high intensity to ambient levels. The question is whether such exposure affects neural functions and behavior.

II. Chemical and cellular changes

Several studies have investigated the effect of RFR on the cholinergic system because of its involvement in learning and wakefulness and animals. Testylier et al. [2002] reported modification of the hippocampal cholinergic system in rats during and after exposure to low-intensity RFR. Bartier et al. [2005] reported that RFR exposure induced structural and biochemical changes in AchE, the enzyme involved in acetylcholine metabolism. Vorobyov et al. [2004] reported that repeated exposure to low-level extremely low frequency-modulated RFR affected baseline and scopolamine-modified EEG in freely moving rats. However, recently Crouzier et al [2007] found no significant change in acetylcholine-induced EEG effect in rats exposed for 24 hours to a 1.8 MHz GSM signal at 1.2 and 9 W/cm^2.

There are several studies on the inhibitory and excitatory neurotransmitters. A decrease in GABA, an inhibitory transmitter, content in the cerebellum was reported by Mausset et al. [2001] after exposure to RFR at 4 W/kg. The same researchers [Maussset-Bonnefont et al., 2004] also reported changes in affinity and concentration of NMDA and GABA receptors in the rat brain after an acute exposure at 6 W/kg. Changes in GABA receptors has also been reported by Wang et al. [2005], and reduced excitatory synaptic activity and number of excitatory synapses in cultured rat hippocampal neurons have been reported by Xu et al. [2006] after RFR exposure. Related to the findings of changes in GABA in the brain is that RFR has been shown to facilitate seizure in rats given subconvulsive doses of picotoxin, a drug that blocks the GABA system [Lopez Martin et al., 2006]. This finding raises the concern that humans with epileptic disorder could be more susceptible to RFR exposure.

Not much has been done on single cell in the brain after RFR exposure. Beason and Semm [2002] reported changes in the amount of neuronal activity by brain cells of birds exposed to GSM signal. Both increase and decrease in firing were observed. Salford et al. [2003] reported cellular damage and death in the brain of rat after acute exposure to GSM signals. Tsurita et al. [2000] reported no significant morphological change in the cerebellum of rats exposed for 2-4 weeks to 1439-MHz TDMA field at 0.25 W/kg. More recently, Joubert et al. [2006, 2007] found no apoptosis in rat cortical neurons exposed to GSM signals in vitro.

III. Learning in Animals

Few animal learning studies have been carried out. All of them reported no significant effect of exposure to cell phone radiation on learning. Bornhausen and Scheingrahen [2000] found no significant change in operant behavior in rats prenatally exposed to a 900-MHz RFR. Sienkiewicz et al. [2000] reported no significant effect on performance in an 8-arm radial maze in mice exposed to a 900-MHz RFR pulsed at 217 Hz at a whole body SAR of 0.05 W/Kg. Dubreuil et al. [2002, 2003] found no significant change in radial maze performance and open-field behavior in rats exposed head only for 45 min to a 217-Hz modulated 900-MHz field at SARs of 1 and 3.5 W/kg. Yamaguichi et al. [2003] reported a change in T-maze performance in the rat only after exposure to a high whole body SAR of 25 W/kg.

IV. Electrophysiology

Studies on EEG and brain evoked-potentials in humans exposed to cellular phone radiation predominantly showed positive effects. The following is a summary of the findings in chronological order. (There are seven related papers published before 1999).

Von Klitzing et al. [1995] were the first to report that cell phone radiation affected EEG alpha activity during and after exposure to cell phone radiation.

Mann and Roschke [1996] reported that cell phone radiation modified REM sleep EEG and shortened sleep onset latency.

Rosche et al. [1997] found no significant change in spectral power of EEG in subjected exposure to cell phone radiation for 3.5 minutes.

Eulitz et al. [1998] reported that cell phone radiation affected brain activity when subjects were processing task-relevant target stimuli and not for irrelevant standard stimuli.

Freude et al. [1998] found that preparatory slow brain potential was significantly affected by cellular phone radiation in certain regions of the brain when the subjects were performing a cognitive complex visual task. The same effects were not observed when subjects were perfoming a simple task.

Urban et al. [1998] reported no significant change in visual evoked potentials after 5 minutes of exposure to cell phone radiation.

Wagner et al. [1998, 2000] reported that cell phone radiation had no significant effect on sleep EEG.

Borbely et al. [1999] reported that the exposure induced sleep and also modified sleep EEG during the non-rapid eye movement (NREM) stage.

Hladky et al. [1999] reported that cell phone use did not affect visual evoked potential.

Freude et al. [2000] confirmed their previous report that cellular phone radiation affected slow brain potentials when subjects are performing a complex task. However, they also reported that the exposure did not significantly affect the subjects in performing the behavioral task. Huber et al. [2000] reported that exposure for 30 minutes to a 900-MHz field at 1 W/kg peak SAR during waking modified EEG during subsequent sleep.

Hietanen et al. [2000] found no abnormal EEG effect, except at the delta band, in subjects exposed for 30 minutes to 900- and 1800-MHz fields under awake, closed-eye condition.

Krause et al. [2000a] reported that cell phone radiation did not affect resting EEG but modified brain activity in subjects performing an auditory memory task.

Krause et al. [2000b] reported that cell phone radiation affected EEG oscillatory activity during a cognitive test. The visual memory task had three different working memory load conditions. The effect was found to be dependent on memory load.

Lebedeva et al. [2000] reported that cell phone radiation affected EEG.

Jech et al. [2001] reported that exposure to cell phone radiation affected visual event-related potentials in narcolepsy patient performing a visual task.

Lebedeva et al. [2001] reported that cell phone radiation affected sleep EEG.

Huber et al [2002] reported that exposure to pulsed modulated RFR prior to sleep affected EEG during sleep. However, effect was not seen with unmodulated field. They also found that the pulsed field altered regional blood flow in the brain of awake subjects.

Croft et al. [2002] reported that radiation from cellular phone altered resting EEG and induced changes differentially at different spectral frequencies as a function of exposure duration.

D'Costa et al. [2003] found EEG effect affected by the radiation within the alpha and beta bands of EEG spectrum.

Huber et al. [2003] reported EEG effect during NREM sleep and the effect was not dependent on the side of the head irradiated. They concluded that the effect involves subcortical areas of the brain that project to both sides of the brain. Dosimetry study shows that the SAR in those area during cell phone use is relatively very low, e.g., 0.1 W/kg at the thalamus. Recently, Aalta et al. [2006], using PET scan imaging, reported a local decrease in regional cerebral blood flow under the antenna in the inferior temporal cortex, but an increase was found in the prefrontal cortex.

Kramarenko et al. [2003] reported abnormal EEG slow waves in awake subjects exposed to cell phone radiation.

Marino et al. [2003] reported an increased randomness of EEG in rabbits.

Hamblin et al. [2004] reported changes in event-related auditory evoked potential in subjects exposed to cellular phone radiation when performing an auditory task. They also found an increase in reaction time in the subjects, but no change in accuracy in the performance.

Hinrich and Heinze [2004] reported a change in early task-specific component of event-related magnetic field in the brain of exposed subjects during a verbal memory encoding task.

Krause et al. [2004] repeated the experiment with auditory memory task [Krause et al., 2000b] and found different effects.

Papageorgiou et al. [2004] reported that cell phone radiation affected male and female EEG differently.

Vorobyov et al. [2004] reported that repeated exposure to modulated microwaves affected baseline and scopolamine-modified EEG in freely moving rats.

Curcio et al. [2005] reported that EEG spectral power affected in the alpha band and the effect was greater when the field was on during EEG recording than when applied before recording.

Hamblin et al. [2005] stated that they could not replicate their previous results on auditory evoked potentials.

Huber et al. [2005] found altered cerebral blood flow in humans exposed to pulsed modulated cell phone radiation. They concluded that, "This finding supports our previous observation that pulse modulation of RF EMF is necessary to induce changes in the waking and sleep EEG, and substantiates the notion that pulse modulation is crucial for RF EMF-induced alterations in brain physiology."

Loughran et al. [2005] reported that exposure to cell phone radiation prior to sleep promoted REM sleep and modified sleep in the first NREM sleep period.

Ferreri et al. [2006] tested excitability of each brain hemisphere by transcranial magnetic stimulation and found that, after 45 minutes of exposure to cellular phone radiation, intracortical excitability was significantly modified with a reduction of inhibition and enhancement in facilitation.

Krause et al. [2006] reported that cell phone radiation affected brain oscillatory activity in children doing an auditory memory task.

Papageorgiou et al. [2006] reported that the radiation emitted by cell phone affects pre-attentive working memory information processing as reflected by changes in P50 evoked potential.

Yuasa et al. [2006] reported no significant effect of cell phone radiation on human somatosensory evoked potentials after 30 minutes of exposure.

Krause et al. [2007] reported effects on brain oscillatory responses during memory task performance. But, they concluded that "The effects on the EEG were, however, varying, unsystematic and inconsistent with previous reports. We conclude that the effects of EMF on brain oscillatory responses may be subtle, variable and difficult to replicate for unknown reasons."

Vecchio et al. [2007] reported that exposure to GSM signal for 45 min modified interhemispheric EEG coherence in cerebral cortical areas.

Hung et al. [2007] reported that after 30 min of exposure to talk-mode mobile phone radiation, sleep latency was markedly and significantly delayed beyond listen and sham modes in healthy human subjects. This condition effect over time was also quite evident in 1-4Hz EEG frontal power, which is a frequency range particularly sensitive to sleep onset.

There is little doubt that electromagnetic fields emitted by cell phones and cell phone use affect electrical activity in the brain. The effect also seems to depend on the mental load of the subject during exposure, e.g., on the complexity of the task that a subject is carrying out. Based on the observation that the two sides of the brain responded similarly to unilateral exposure, Huber et al. [2003] deduced that the EEG effect originated from subcortical areas of the brain. Dosimetry calculation indicates that the SAR in such areas could be as low as 0.1 W/kg.

However, the behavioral consequences of these neuroelectrophysiological changes are not always predictable. In several studies (e.g., Freude et al., 2000; Hamblin et al, 2004), cell phone radiation-induced EEG changes were not accompanied by a change in psychological task performance of the subjects. The brain has the flexibility to accomplish the same task by different means and neural pathways. Does cell phone radiation alter information-processing functions in the brain as reported previously with RFR exposure [Wang and Lai, 2000]? In the next section, we will look at the effects of cell phone radiation exposure on cognitive functions in humans.

V. Cognitive functions

Again, findings are listed below in chronological order.

Preece et al. [1999] were the first to report an increase in responsiveness, strongly in the analogue and less in the digital cell phone signal, in choice reaction time.

Cao et al. [2000] showed that the average reaction time in cell phone users was significantly longer than that in control group in psychological tests. The time of use was negatively associated with corrected reaction number.

Koivisto et al. [2000a, b] reported a facilitation of reaction in reaction time tasks during cell phone radiation exposure. In a working memory test, exposure speeded up response times when the memory load was three items but no significant effect was observed with lower loads.

Jech et al. [2001] reported that cell phone radiation may suppress the excessive sleepiness and improve performance while solving a monotonous cognitive task requiring sustained attention and vigilance in narcolepsy patients.

Lee et al. [2001] reported a facilitation effect of cell phone radiation in attention functions.

Edelstyn and Oldershaw [2002] found in subjects given 6 psychological tests a significant difference in three tests after 5 min of exposure. In all cases, performance was facilitated following cell phone radiation exposure.

Haarala et al. [2003] found no significant effect of cell phone radiation on the reaction time and response accuracy of subjects performed in 9 cognitive tasks.

Lee et al. [2003] reported that the facilitation effect of cell phone radiation on attention functions is dose (exposure duration)-dependent.

Smythe and Costall [2003] using a word learning task, found that male subjects made significantly less error than unexposed subject. However, the effect was not found in female subjects. (Papageorgiou et al. [2004] also reported that cell phone radiation affected male and female EEG differently.)

Curcio et al. [2004] found in subjects tested on four performance tasks, an improvement of both simple- and choice-reaction times. Performance needed a minimum of 25 min of EMF exposure to show significant changes.

Haarala et al. [2004] reported that they could not replicate their previous results [Koivisto ret al., 2000a] on the effect of cell phone radiation on short-term memory.

Maier et al. [2004] found that subjects exposed to GSM signal showed worse results in their auditory discrimination performance as compared with control conditions.

Basset et al. [2005] reported no significant effect of daily cell phone use on a battery of neuropsychological tests screening: information processing, attention capacity, memory function, and executive function. The authors concluded that "…our results indicate that daily MP use has no effect on cognitive function after a 13-h rest period."

Haarala et al [2005] reported that 10-14 year old children's cognitive functions were not affected by cell phone radiation exposure.

Preece et al. [2005] concluded that, "this study on 18 children did not replicate our earlier finding in adults that exposure to microwave radiation was associated with a reduction in reaction time." They speculated that the reason for the failure to replicate was because a less powerful signal was used in this study.

Schmid et al. [2005] reported no significant effect of cell phone radiation on visual perception.

Eliyaku et al. [2006] reported in subjects given 4 cognitive tasks that exposure of the left side of the brain slowed down the left-hand response time in three of the four tasks.

Keetley et al. [2006] tested 120 subjects on 8 neuropsychological tests and concluded that cell phone emissions "improve the speed of processing of information held in working memory."

Russo et al. [2006] reported that GSM or CW signal did not significantly affect a series of cognitive tasks including a simple reaction task, a vigilance task, and a subtraction task.

Terao et al. [2006] found no significant effect of cell phone use on the performance of visuo-motor reaction time task in subjects after 30 minutes of exposure.

Haarala et al. [2007] concluded that 'the current results indicate that normal mobile phones have no discernible effect on human cognitive function as measured by behavioral tests.'

Terao et al. [2007] reported no significant effect of a 30-min exposure to mobile phone radiation on the performance of various saccade tasks (visually-guided, gap, and memory-guide), suggesting that the cortical processing for saccades and attention is not affected by the exposure.

Cinel et al. [2007] reported that acute exposure to mobile phone RF EMF did not affect performance in the order threshold task.

Thus, a majority of the studies (13/23) showed that exposure to cell phone could affect cognitive functions and affect performance in various behavioral tasks. Interestingly, most of these studies showed a facilitation and improvement in performance. Only the studies of Cao et al. [2000], Maier et al. [2004] and Eliyaku et al. [2006] reported a performance deficit. (It may be significant to point out that of the 10 studies that reported no significant effect, 6 of them were funded by the cell phone industry and one [Terao et al., 2006] received partial funding from the industry.)

VI. Auditory effect

Since the cell phone antenna is close to the ear during use, a number of studies have been carried out to investigate the effect of cell phone radiation on the auditory system and its functions. Kellenyi et al. [1999] reported a hearing deficiency in the high frequency range in subjects after 15 minutes of exposure to cell phone radiation. Mild hearing loss was reported by Garcia Callejo et al. [2005], Kerckhanjanarong et al [2005] and Oktay and Dasdag [2006] in cell phone users. However, these changes may not be related to exposure to electromagnetic fields. Recently, Davidson and Lutman [2007] reported no chronic effects of cell phone usage on hearing, tinnitus and balance in a student population.

Auditory-evoked responses in the brain have been studied. Kellenyi et al. [1999], in addition to hearing deficiency, also reported a change in auditory brainstem response in their subjects. However, no significant effect on brainstem and cochlear auditory responses were found by Arai et al.[2003], Aran et al. [2004], and Sievert et al. [2005]. However, Maby et al. [2004, 2005, 2006] reported that GSM electromagnetic fields modified human auditory cortical activity recorded at the scalp.

Another popular phenomenon studied in this aspect is the distorted product otoacoustic emission, a measure of cochlear hair cell functions. Grisanti et al. [1998] first reported a change in this measurement after cell phone use. Subsequent studies by various researchers using different exposure times and schedules failed to find any significant effect of cell phone radiation [Aren et al. 2004; Galloni et al., 2005 a,b; Janssen et al., 2005; Kizilay et al, 2003; Marino et al., 2000; Monnery et al., 2004; Mora et al., 2006; Ozturan et al., 2002; Parazzini et al., 2005; Uloziene et al., 2005].

There have been reports suggesting that people who claimed to be hypersensitive to EMF have higher incidence of tinnitus [Cox, 2004: Fox, 2004; Holmboe and Johansson, 2005]. However, data from the physiological studies described above do not indicate that EMF exposure could cause tinnitus.

VII. Human subjective effects

Abdel-Rassoul G, El-Fateh OA, Salem MA, Michael A, Farahat F, El-Batanouny M, Salem E. Neurobehavioral effects among inhabitants around mobile phone base stations. Neurotoxicology. 28:434-440, 2007.

Al-Khlaiwi T, Meo SA. Association of mobile phone radiation with fatigue, headache, dizziness, tension and sleep disturbance in Saudi population. Saudi Med J. 25(6):732-736, 2004.

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Wilen J, Sandstrom M, Hansson Mild K. Subjective symptoms among mobile phone users-A consequence of absorption of radiofrequency fields? Bioelectromagnetics 24(3):152-159, 2003.

Wilen J, Johansson A, Kalezic N, Lyskov E, Sandstrom M. Psychophysiological tests and provocation of subjects with mobile phone related symptoms. Bioelectromagnetics 27:204-214, 2006.

The possible existence of physical symptoms from exposure to RFR from various sources including cell phones, cell towers and wireless systems has been a topic of significant public concern and debate. This is an issue that will require additional attention. Symptoms that have been reported include: sleep disruption and insomnia, fatigue, headache, memory loss and confusion, tinnitus, spatial disorientation and dizziness. However, none of these effects has been studied under controlled laboratory conditions. Thus, whether they are causally related to RFR exposure is unknown.

VIII. Summary and Discussion

A. Research data are available suggesting effects of RFR exposure on neurological and behavioral functions. Particularly, effects on neurophysiological and cognitive functions are quite well established. Interestingly, most of the human studies showed an enhancement of cognitive function after exposure to RFR, whereas animals studied showed a deficit. However, research on electrophysiology also indicates that effects are dependent on the mental load of the subjects during exposure. Is this because the test-tasks used in the animal studies are more complex or the nervous system of non-human animals can be easier overloaded? These point to an important question on whether RFR-induced cognitive facilitation still occurs in real life situation when a person has to process and execute several behavioral functions simultaneously. Generally speaking, when effects were observed, RFR disrupted behavior in animals, such as in the cases of behaviors to adapt to changes in the environment and learning. This is especially true when the task involved complex responses. In no case has an improvement in behavior been reported in animals after RFR exposure. It is puzzling that only disruptions in behavior by RFR exposure are reported in non-human animals. In the studies on EEG, both excitation and depression have been reported after exposure to RFR. If these measurements can be considered as indications of electrophysiological and behavioral arousal and depression, improvement in behavior should occur under certain conditions of RFR exposure. This is now reported in humans exposed to cell phone radiation.

B. On the other hand, one should be very careful in extrapolating neurological/behavioral data from non-human in vivo experiments to the situation of cell phone use in humans. The structure and anatomy of animal brains are quite different from those of the human brain. Homologous structures may not be analogous in functions. Differences in head shape also dictate that different brain structures would be affected under similar RF exposure conditions. Thus, neurological data from human studies should be more reliable indicators of cell phone effects.

C. Another consideration is that most of the studies carried out so far are short-term exposure experiments, whereas cell phone use causes long-term repeated exposure of the brain. Depending on the responses studied in neurological/behavioral experiments, several outcomes have been reported after long term exposure: (1) an effect was observed only after prolonged (or repeated) exposure, but not after one period of exposure; (2) an effect disappeared after prolonged exposure suggesting habituation; and (3) different effects were observed after different durations of exposure. All of these different responses reported can be explained as being due to the different characteristics of the dependent variable studied. These responses fit the pattern of general responses to a 'stressor'. Indeed, it has been proposed that RFR is a 'stressor' (e.g., see http://www.wave-guide.org/library/lai.html). Chronic stress could have dire consequences on the health of a living organism. However, it is difficult to prove that an entity is a stressor, since the criteria of stress are not well defined and the caveat of stress is so generalized that it has little predictive power on an animal's response.

D. From the data available, in general, it is not apparent that pulsed RFR is more potent than continuous-wave RFR in affecting behavior in animals. Even though different frequencies and exposure conditions were used in different studies and hardly any dose-response study was carried out, there is no consistent pattern that the SARs of pulsed RFR reported to cause an effect are lower than those of continuous-RFR. This is an important consideration on the possible neurological effects of exposure to RFR during cell phone use, since cell phones emit wave of various forms and characteristics.

E. Thermal effect cannot be discounted in the effects reported in most of the neurological/behavioral experiments described above. Even in cases when no significant change in body or local tissue temperature was detected, thermal effect cannot be excluded. An animal can maintain its body temperature by actively dissipating the heat load from the radiation. Activation of thermoregulatory mechanisms can lead to neurochemical, physiological, and behavioral changes. However, several points raised by some experiments suggest that the answer is not a simple one. They are: (a) 'Heating controls' do not produce the same effect of RFR; (b) Window effects are reported; (c) Modulated or pulsed RFR is more effective in causing an effect or elicits a different effect when compared with continuous-wave radiation of the same frequency.

F. It is also interesting to point out that in most of the behavioral experiments, effects were observed after the termination of RFR exposure. In some experiments, tests were made days after exposure. This suggests a persistent change in the nervous system after exposure to RFR.

G. In many instances, neurological and behavioral effects were observed at a SAR less than 4 W/kg. This directly contradicts the basic assumption of the IEEE guideline criterion.

H. A question that one might ask is whether different absorption patterns in the brain or body could elicit different biological responses in an animal. If this is positive, possible outcomes from the study of bioelectromagnetics research are: (a) a response will be elicited by some exposure conditions and not by others, and (b) different response patterns are elicited by different exposure conditions, even though the average dose rates in the conditions are equal. These data indicate that energy distribution in the body and other properties of the radiation can be important factors in determining the outcome of the biological effects of RFR.

I. Even though the pattern or duration of RFR exposure is well-defined, the response of the biological system studied will still be unpredictable if we lack sufficient knowledge of the response system. In most experiments on the neurological effects of RFR, the underlying mechanism of the dependent variable was not fully understood. The purpose of most of the studies was to identify and characterize possible effects of RFR rather than the underlying mechanisms responsible for the effects. Understanding the underlying mechanism is an important criterion in understanding an effect.

J. Another important consideration in the study of the central nervous system should be mentioned here. It is well known that the functions of the central nervous system can be affected by activity in the peripheral nervous system. This is especially important in the in vivo experiments when the whole body is exposed. However, in most experiments studying the effects of RFR on the central nervous system, the possibility of contribution from the peripheral nervous system was not excluded in the experimental design. Therefore, caution should be taken in concluding that a neurological effect resulted solely from the action of RFR on the central nervous system.

K. In conclusion, the questions on the neurological effects (and biological effects, in general) of RFR and the discrepancies in research results in the literature can be resolved by (a) a careful and thorough examination of the effects of the different radiation parameters, and (b) a better understanding of the underlying mechanisms involved in the responses studied. With these considerations, it is very unlikely that the neurological effects of RFR can be accounted for by a single unifying neural mechanism.

L. Finally, does disturbance in behavior have any relevance to health? The consequence of a behavioral deficit is situation dependent and may not be direct. It probably does not matter if a person is playing chess and RFR in his environment causes him to make a couple of bad moves. However, the consequence would be much more serious if a person is flying an airplane and his response sequences are disrupted by RFR radiation.

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Appendix 9-A

NEUROLOGICAL EFFECTS OF RADIOFREQUENCY ELECTROMAGNETIC RADIATION in "Advances in Electromagnetic Fields in Living Systems, Vol. 1," J.C. Lin (ed.), Plenum Press, New York. (1994) pp. 27-88

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INTRODUCTION

Many reports in the literature have suggested the effect of exposure to radiofrequency electromagnetic radiation (RFR) (10 kHz-300,000 MHz) on the functions of the nervous system. Such effects are of great concern to researchers in bioelectromagnetics, since the nervous system coordinates and controls an organism's responses to the environment through autonomic and voluntary muscular movements and neurohumoral functions. As it was suggested in the early stages of bioelectromagnetics research, behavioral changes could be the most sensitive effects of RFR exposure. At the summary of session B of the proceedings of an international symposium held in Warsaw, Poland, in 1973, it was stated that "The reaction of the central nervous system to microwaves may serve as an early indicator of disturbances in regulatory functions of many systems" [Czerski et al., 1974].

Studies on the effects of RFR on the nervous system involve many aspects: morphology, electrophysiology, neurochemistry, neuropsychopharmacology, and psychology. An obvious effect of RFR on an organism is an increase in temperature in the tissue, which will trigger physiological and behavioral thermal regulatory responses. These responses involve neural activities both in the central and peripheral nervous systems. The effects of RFR on thermoregulation have been extensively studied and reviewed in the literature [Adair, 1983; Stern, 1980]. The topic of thermoregulation will not be reviewed in this chapter. Since this paper deals mainly with the effects of RFR on the central nervous system, the effect on neuroendocrine functions also will not be reviewed here. It is, however, an important area of research since disturbances in neuroendocrine functions are related to stress, alteration in immunological responses, and tumor development [Cotman et al., 1987; Dunn, 1989; Plotnikoff et al., 1991]. Excellent reviews of research on this topic have been written by Lu et al.[1980] and Michaelson and Lin [1987].

In order to give a concise review of the literature on the effects of RFR on neural functions, we have to first understand the normal functions of the nervous system.

PRINCIPLES OF NEURAL FUNCTIONS

The nervous system is functionally composed of nerve cells (neurons) and supporting cells known as glia. In higher animal species, it is divided into the central and peripheral nervous systems. The central nervous system consists of the brain and the spinal cord and is enveloped in a set of membranes known as the meninges. The outer surface as well as the inner structures of the central nervous system are bathed in the cerebrospinal fluid (CSF) that fills the ventricles of the brain and the space at the core of the spinal cord.

The brain is generally subdivided into regions (areas) based on embryological origins. The anterior portion of the neural tube, the embryonic tissue from which the nervous system is developed, has three regions of expansion: the forebrain, midbrain, and hindbrain. From the forebrain, the cerebral hemispheres and the diencephalon will develop. The diencephalon consists of the thalamus, epithalamus, subthalamus, and hypothalamus. The midbrain remains mostly unchanged from the original structure of the neural tube; however, two pairs of structures, the superior and inferior colliculi, develop on its dorsal surface. These are parts of the visual and auditory systems, respectively. The hindbrain develops into the medulla, pons, and cerebellum.

The thalamus of the diencephalon is divided into various groups of cells (nuclei). Some of these nuclei are relays conveying sensory information from the environment to specific regions of the cerebral cortex, such as the lateral and medial geniculate nuclei that relay visual and auditory information, respectively, from the eyes and ears to the cerebral cortex. Other nuclei have more diffuse innervations to the cerebral cortex. The hypothalamus is involved in many physiological regulatory functions such as thermoregulation and control of secretion of hormones.

The cerebral hemispheres consist of the limbic system (including the olfactory bulbs, septal nucleus, amygdala, and hippocampus), the basal ganglia (striatum), and the cerebral cortex. The limbic system serves many behavioral functions such as emotion and memory. The striatum is primarily involved in motor controls and coordination. The cerebral cortex especially in the higher animal species is divided into regions by major sulci: frontal, parietal, temporal, and occipital cortex, etc. The function of some regions can be traced to the projection they receive from the thalamus, e.g., the occipital cortex (visual cortex) processes visual information it receives from the lateral geniculate nucleus of the thalamus and the temporal cortex (auditory cortex) receives auditory information from the medial geniculate nucleus. There are other cortical areas, however, known as secondary sensory areas and 'association' cortex that receive no specific thalamic innervations. One example of the association cortical areas is the prefrontal cortex, which is supposed to subserve higher behavioral functions, e.g., cognition.

The basic design of the central nervous system is similar among species in the phylogenetic scale; however, there are differences in the details of structure among species. Most of the brain regions mentioned in the above sections have been studied in bioelectromagnetics research to a various extent.

On the neurochemical level, neurons with similar biochemical characteristics are usually grouped together to form a nucleus or ganglion. Information is transmitted by electrochemical means via fibers (axons) protruding from the neuron. In addition to making local innervations to other neurons within the nucleus, nerve fibers from the neurons in a nucleus are also grouped into bundles (pathways) that connect one part of the brain to another. Information is generally passed from one neuron to another via the release of chemicals. These chemicals are called neurotransmitters or neuromodulators depending upon their functions. Many neurotransmitters have been identified in the central nervous system. Some are small molecules such as acetylcholine, norepinephrine, dopamine, serotonin, and γ–amino-butyric acid (GABA), whereas the others are polypeptides and proteins such as the endogenous opioids, substance-P, etc. Effects of RFR on most of these neurotransmitters have been investigated. Nerve fibers in a pathway usually release the same neurotransmitter. The anatomy of some of these neurotransmitter pathways are well studied such as those of dopamine, norepinephrine, serotonin, and acetylcholine.

After a neurotransmitter is released, it passes a space gap (synapse) between two adjacent cells and reacts with a molecule known as "receptor" at the cell membrane of the receiving (postsynaptic) cell. Such a reaction is usually described as analogous to the action of the key and lock. A particular neurotransmitter can only bind to its specific receptor to exert an effect. Binding of the neurotransmitter to a receptor triggers a series of reactions that affect the postsynaptic cell. Properties of the receptors can be studied by the receptor-ligand binding technique. Using this method the concentration and the binding affinity to the neurotransmitter of the receptors in a neural tissue sample can be determined.

Pharmacologically, one can affect neural functions by altering the events of synaptic transmission by the administration of a drug. Drugs can be used to decrease or increase the release of neurotransmitters or affect the activity of the receptors. Many drugs exert their effects by binding to neurotransmitter receptors. Drugs which have actions at the receptors similar to those of the natural neurotransmitters are called agonists, whereas drugs which block the receptors (thus blocking the action of the endogenous neurotransmitters) are known as antagonists. The property of antagonists provides a powerful conceptual tool in the study of the functions of the nervous system. Neural functions depend on the release of a particular type of neurotransmitter. If a certain physiological or behavioral function is blocked by administration of a certain antagonist to an animal, one could infer that the particular neurotransmitter blocked by the antagonist is involved in the function. In addition, since neurons of the same chemical characteristics are grouped together into pathways in the nervous system, from the information obtained from the pharmacological study, one can speculate on the brain areas affected by a certain treatment such as RFR.

The activity in the synapses is dynamic. In many instances as a compensatory response to changes in transmission in the synapses, the properties (concentration and/or affinity) of the receptors change. Generally, as a result of repeated or prolonged increase in release of a neurotransmitter, the receptors of that neurotransmitter in the postsynaptic cells decrease in number or reduce their binding affinity to the neurotransmitter. The reverse is also true, i.e., increase in concentration or binding affinity of the receptors occurs after prolonged or repeated episodes of decreased synaptic transmission. Such changes could have important implications on an animal's functional state. The changes in neurotransmitter receptors enable an animal to adapt to the repeated perturbation of function. On the other hand, since changes in receptor properties can last for a long time (days to weeks), an animal's normal physiological and behavioral functions will be altered by such changes.

The central nervous system of all vertebrates is enveloped in a functional entity known as the blood-brain barrier, due to the presence of high-resistance tight junctions between endothelial cells in the capillaries of the brain and spinal cord. The blood-brain barrier is impermeable to hydrophilic (polar) and large molecules and serves as a protective barrier for the central nervous system against foreign and toxic substances. Many studies have been carried out to investigate whether RFR exposure affects the permeability of the blood-brain barrier.

Drugs can be designed that cannot pass through the blood-brain barrier and, thus, they can only affect the peripheral nervous system. Using similar antagonists that can and cannot pass through the blood-brain barrier, one can determine whether an effect of an entity such as RFR is mediated by the central or peripheral nervous system. On the other hand, drugs can be directly injected into the central nervous system (thus, by-passing the blood-brain barrier) to investigate the roles of neural mechanisms inside the brain on a certain physiological or behavioral function.

Changes in neurochemical functions lead to changes in behavior in an animal. Research has been carried out to investigate the effects of RFR exposure on spontaneous and learned behaviors. Motor activity is the most often studied spontaneous behavior. Alteration in motor activity of an animal is generally considered as an indication of behavioral arousal. For learned behavior, conditioned responses were mostly studied in bioelectromagnetics research. The behavior of an animal is constantly being modified by conditioning processes, which connect behavioral responses with events (stimuli) in the environment. Two types of conditioning processes have been identified and they are known as classical and operant conditioning. In classical conditioning, a 'neutral' stimulus that does not naturally elicit a certain response is repeatedly being presented in sequence with a stimulus that does elicit that response. After repeated pairing, presentation of the neutral stimulus (now the conditioned stimulus) will elicit the response (now the conditioned response). Interestingly, the behavioral control probability of the conditioned stimulus is shared by similar stimuli, i.e., presentation of a stimulus similar to the conditioned stimulus can also elicit the conditioned response. The strength and probability of occurrence of the conditioned response depends on the degree of similarity between the two stimuli. This is known as "stimulus generalization."

A paradigm of classical conditioning used in bioelectromagnetics research is the "conditioned suppression" procedure. Generally, in this conditioning process, an aversive stimulus (such as electric shock, loud noise) follows a warning signal. After repeated pairing, the presentation of the warning signal alone can stop or decrease the on-going behavior of the animal. The animal usually "freezes" for several minutes and shows emotional responses like defecation and urination. Again, stimulus generalization to the warning signal can occur.

Operant (or instrumental) conditioning involves a change in the frequency or probability of a behavior by its consequences. Consequences which increase the rate of the behavior are known as "reinforcers". Presentation of a "positive reinforcer", e.g., availability of food to a hungry animal, increases the behavior leading to it. On the other hand, removal of a "negative reinforcer", e.g., an electric shock, also leads to an increase of the behavior preceding it. Presentation of an aversive stimulus will decrease the probability of the behavior leading to it. In addition, removal of a positive reinforcer contingent upon a response will also decrease the probability of further response. Thus, both positive and negative reinforcers increase the probability of a response leading to them, and punishment (presentation of an aversive stimulus or withdrawal of a positive reinforcer) decreases the occurrence of a response. The terms used to describe a consequence are defined by the experimental procedures. The same stimulus can be used as a "negative reinforcer" to increase a behavior or as a punisher to decrease the behavior.

An interesting aspect of behavioral conditioning is the schedule on which an animal is reinforced (schedule-controlled behavior). An animal can be reinforced for every response it emits; however, it can also be reinforced intermittently upon responding. Intermittent reinforcement schedules generally consist of the following: reinforcement is presented after a fixed number of responses (fixed ratio), a fixed period of time (fixed interval), or a variable number of responses (variable ratio) or interval of time (variable interval) around an average value. The intermittent reinforcement schedules have a profound effect on the rate and pattern of responding. The variable schedules generally produce a steadier responding rate than the fixed schedules. A post-reinforcement pulse is associated with the fixed schedules when the rate of responding decreases immediately after a reinforcement and then increases steadily. Ratio schedules generally produce a higher responding rate than interval schedules. Another simple reinforcement schedule commonly used in bioelectromagnetics research is the differential reinforcement of a low rate of responding (DRL). In this schedule, a reinforcement only follows a response separated from the preceding response by a specific time interval. If the animal responds within that time, the timer will be reset and the animal has to wait for another period of time before it can elicit a reinforceable response. The DRL schedule, dependent of the time interval set, produces a steady but low rate of responding. Compound schedules, consisting of two or more of the above schedule types, can also be used in conditioning experiments to control behavior. A multiple schedule is one in which each component is accompanied by a discriminatory stimulus, e.g., a white light when a fixed interval schedule is on and a green light when a variable interval schedule is on. The multiple schedule paradigm is widely used in pharmacological research to compare the effect of a drug on the patterns of response under different schedules in the same individual. A mixed schedule is a multiple schedule with no discriminative stimulus associated with each schedule component. Thus, a multiple schedule produces descrete patterns of responding depending on the currently active schedule, whereas a mixed schedule produces a response pattern that is a blend of all the different components. A tandem schedule consists of a sequence of schedules. Completion of one schedule leads to access to the next schedule, with no reinforcement presented until the entire sequence of schedules is completed. A chained schedule is a tandem schedule with each component accompanied by a discriminatory stimulus. Other more complicated combinations of schedules can be used in conditioning experiments. These compound schedules pose increased difficulties in an animal's ability to respond and make the performance more sensitive to the disturbance of experimental manipulations such as RFR.

In operant discrimination learning, an animal learns to elicit a certain response in the presence of a particular environmental stimulus, e.g., light, and is rewarded after the response, whereas no reinforcement is available in the absence of the stimulus or in the presence of another stimulus, e.g., tone. In this case, generalization to similar stimuli can also occur.

Another popular paradigm used in the research on the behavioral effects of RFR is escape and avoidance learning. In escape responding an animal elicits a response immediately when an aversive stimulus, e.g., electric foot-shock, is presented in order to escape from it or to turn it off. In avoidance learning an animal has to make a certain response to prevent the onset of an aversive stimulus. The avoidance can be a signalled avoidance-escape paradigm in which a stimulus precedes the aversive stimulus. On the other hand, the aversive stimulus can be nonsignalled. In this case the animal has to respond continuously to postpone the onset of the aversive stimulus, otherwise it will be presented at regular intervals. This paradigm is also known as "continuous-avoidance." It was speculated that avoidance learning was reinforced by reduction of a conditioned fear reaction [Mowrer, 1939; Solomon and Wynne, 1954]. In escape-avoidance learning both classical and operant conditioning processes are involved.

Use of reinforcement-schedules can generate orderly and reproducible behavioral patterns in animals, and thus, allows a systematic study of the effect of an independent variable, such as RFR. However, the underlying mechanisms by which different schedules affect behavior are poorly understood. The significance of studying schedule-controlled behavior has been discussed by Jenkins [1970] and Reynolds [1968]. In addition, de Lorge [1985] has written a concise and informative review and comments on the use of schedule-controlled behavior in the study of the behavioral effects of RFR.

In the following review on the effects of RFR on the central nervous system the concepts described above on the functions of the nervous system will apply.

EFFECTS OF RADIOFREQUENCY RADIATION ON THE MORPHOLOGY OF THE CENTRAL NERVOUS SYSTEM

Cellular Morphology

Radiofrequency radiation-induced morphological changes of the central nervous system are not expected except under relatively high intensity or prolonged exposure to the radiation. Such changes are not a necessary condition for alteration in neural functions after exposure to RFR. Early Russian studies [Gordon, 1970; Tolgskaya and Gordon, 1973] reported morphological changes in the brain of rats after 40 min of exposure to 3000- or 10000-MHz RFR at power densities varying from 40-100 mW/cm^2 (rectal temperature increased to 42-45 ^oC). Changes included hemorrhage, edema, and vacuolation formation in neurons. In these studies, changes in neuronal morphology were also reported in the rat brain after repeated exposure to RFR of lower power densities (3000 MHz, thirty-five 30-min sessions, <10 mW/cm^2, SAR 2 W/kg). Changes included neuronal cytoplasmic vacuolation, swelling and beading of axons, and a decrease in the number of dendritic spines. Albert and DeSantis [1975] also reported swollen neurons with dense cytoplasm and decreased rough endoplasmic reticulum and polyribosomes, indicative of decreased protein synthesis, in the hypothalamus and subthalamic region of the brain of hamsters exposed for 30 min to 24 h to continuous-wave 2450-MHz RFR at 50 mW/cm^2 (SAR 15 W/kg). No observable effect was seen in the thalamus, hippocampus, cerebellum, pons, and spinal cord. Recovery was seen at 6-10 days postexposure. In the same study, vacuolation of neurons was also reported in the hypothalamus of hamsters exposed to 2450-MHz RFR at 24 mW/cm^2 (SAR 7.5 W/kg) for 22 days (14 h/day). Similar effects of acute exposure were observed in a second study [Albert and DeSantis, 1976] when hamsters were exposed for 30-120 min to continuous-wave 1700-MHz RFR at either 10 (SAR 3 W/kg) or 25 mW/cm^2 (SAR 7.5 W/kg). The effects persisted even at 15 days postexposure.

Baranski [1972] reported edema and heat lesions in the brain of guinea pigs exposed in a single 3-h session to 3000-MHz RFR at a power density of 25 mW/cm^2 (SAR 3.75 W/kg). After repeated exposure (3 h/day for 30 days) to similar radiation, myelin degeneration and glial cell proliferation were reported in the brains of exposed guinea pigs (3.5 mW/cm^2, SAR 0.53 W/kg) and rabbits (5 mW/cm^2, SAR 0.75 W/kg). Pulsed (400 pps) RFR produced more pronounced effects in the guinea pigs than continuous-wave radiation of the same power density. Switzer and Mitchell [1977] also reported an increase in myelin figures (degeneration) of neurons in the brain of rats at 6 weeks after repeated (5 h/day, 5 day/week for 22 weeks) exposure to continuous-wave 2450-MHz RFR (SAR 2.3 W/kg). In another study [McKee et al., 1980], Chinese hamsters were exposed to continuous-wave 1700-MHz RFR at 10 or 25 mW/cm^2 (SARs 5 and 12.5 W/kg) for 30-120 min. Abnormal neurons were reported in the hypothalamus, hippocampus, and cerebral cortex of the animals after exposure. In addition, platelet aggregation and occlusion of some blood vessels in the brain were also reported.

Two studies investigated the effects of perinatal exposure to RFR on the development of Purkinje cells in the cerebellum. In the first study [Albert et al., 1981a], pregnant squirrel monkeys were exposed to continuous-wave 2450-MHz RFR (3 h/day, 5 days/week) at a power density of 10 mW/cm^2 (SAR 3.4 W/kg) and the offspring were similarly exposed for 9.5 months after birth. No significant change was observed in the number of Purkinje cells in the uvula areas of the cerebellum of the exposed animals compared to that of controls. In the second study, Albert et al. [1981b] studied the effects of prenatal, postnatal, and pre- and postnatal-RFR exposure on Purkinje cells in the cerebellum of the rat. In the prenatal exposure experiment, pregnant rats were exposed from 17-21 days of gestation to continuous-wave 2450-MHz RFR at 10 mW/cm^2 (SAR 2W/kg) for 21 h/day. The offspring were studied at 40 days postexposure. A decrease (-26%) in the concentration of Purkinje cells was observed in the cerebellum of the prenatally RFR-exposed rats. In the pre- and postnatal-exposure experiment, pregnant rats were exposed 4 h/day between the 16-21 days of gestation and their offspring were exposed for 90 days to continuous-wave 100-MHz RFR at 46 mW/cm^2 (SAR 2.77 W/kg). Cerebellum morphology was studied at 14 months postexposure. A 13% decrease in Purkinje cell concentration was observed in the RFR-exposed rats. The changes observed in the pre- and perinatally-exposed rats seemed to be permanent, since the animals were studied more than a month postexposure. In the postnatal exposure experiment, 6-day old rat pups were exposed 7 h/day for 5 days to 2450-MHz RFR at 10 mW/cm^2 and their cerebella were studied immediately or at 40 days after exposure. A 25% decrease in Purkinje cell concentration was found in the cerebellum of rats studied immediately after exposure, whereas no significant effect was observed in the cerebellum at 40 days postexposure. Thus, the postnatal exposure effect was reversible. The authors suggested that RFR may affect the proliferative activity and migrational process of Purkinje cells during cerebellar development. In a further study [Albert and Sherif, 1988], 1- or 6-day old rat pups were exposed to continuous-wave 2450-MHz RFR for 5 days (7 h/day, 10 mW/cm^2, SAR 2W/kg). Animals were killed one day after the exposure and morphology of their cerebellum was studied. The authors reported two times the number of deeply stained cells with dense nucleus in the external granular layer of the cerebellum. Examination with an electron microscope showed that the dense nuclei were filled with clumped chromatin. Extension and disintegration of nucleus, ruptured nuclear membrane, and vacuolization of the cytoplasm were observed in these cells. Some cells in the external granular layer normally die during development of the cerebellum; therefore, these data showed that postnatal RFR exposure increased the normal cell death. In the same study, disorderly arrays of rough endoplasmic reticulum were observed in the Purkinje cells of the exposed animals indicating an altered metabolic state in these cells.

Blood-Brain Barrier

Intensive research effort was undertaken to investigate whether RFR affected the permeability of the blood-brain barrier [Albert, 1979b; Justesen, 1980]. The blood-brain barrier blocks the entry of large and hydrophilic molecules in the general blood circulation from entering the central nervous system. Its permeability was shown to be affected by various treatments, e.g., electroconvulsive shock [Bolwig, 1988]. Variable results on the effects of RFR on blood-brain barrier permeability have been reported. A reason for this could be due to the difficulties in measuring and quantifying the effect [Blasberg, 1979].

Frey et al. [1975] reported an increase in fluorescein in brain slices of rats injected with the dye and exposed for 30 min to continuous-wave 1200-MHz RFR (2.4 mW/cm^2, SAR 1.0 W/kg) as compared with control animals. The dye was found mostly in the lateral and third ventricles of the brain. A similar but more pronounced effect was observed when the animals were exposed to pulsed 1200-MHz RFR at an average power density of 0.2 mW/cm^2. These data were interpreted as an indication of an increase in permeability of the blood-brain barrier, since fluorescein injected systemically does not normally permeate into the brain. On the other hand, Merritt et al. [1978] did not observe a significant change in the permeability of fluorescein-albumin into the brain of rats exposed to a similar dose-rate of RFR (1200 MHz, either continuous-wave or pulsed, 30 min, 2-75 mW/cm^2); however, an increase in permeability was observed, if the body temperature of the animal was raised to 40 ^{o}C either by RFR or convective heating. In addition, no significant change in permeability of mannitol and inulin to the brain was reported in this experiment after RFR exposure.

Chang et al. [1982] studied in the dog the penetration of ^{131}I-labelled albumin into the brain. The head of the dog was irradiated with 1000-MHz continuous-wave RFR at 2, 4, 10, 30, 50, or 200 mW/cm^2 and the tracer was injected intravenously. Radioactivity in the blood and cerebrospinal fluid (CSF) was determined at regular time intervals postinjection. An increase in the ratio of radioactivity in the CSF versus that in the blood was considered as an indication of entry of the labelled albumin that normally does not cross the blood-brain barrier. At 30 mW/cm^2, 4 of the 11 dogs studied showed a significant increase in the ratio compared to that of sham-exposed animals, whereas no significant difference was seen at the other power densities. The authors suggested a possible 'power window' effect.

Lin and Lin [1980] reported no significant change in the permeability of sodium fluorescein and Evan's blue into the brain of rats with focal exposure at the head for 20 min to pulsed 2450-MHz RFR at 0.5-1000 mW/cm^2 (local SARs 0.04-80 W/kg), but an increase was reported after similar exposure of the head at an SAR of 240 W/kg [Lin and Lin, 1982]. The brain temperature under the latter exposure condition was 43 ^{o}C. In a further study, by the same laboratory, Goldman et al. [1984] used ^{86}Rb as the tracer to study the permeability of the blood-brain barrier after RFR exposure. The tracer was injected intravenously to rats after 5, 10, or 20 min of exposure to 2450-MHz pulsed RFR (10 μs pulses, 500 pps) at an average power density of 3 W/cm^2 (SAR 240 W/kg) on the left side of the head. Brain temperature was increased to 43 ^{o}C. The ^{86}Rb uptake in the left hemisphere of the brain was studied. Increase in uptake was detected in the hypothalamus, striatum, midbrain, dorsal hippocampus, and occipital and parietal cortex at 5 min postexposure. Increased uptake of the tracer in the cerebellum and superior colliculus was also observed at 20 min after exposure. That increase in brain temperature played a critical role in the effect of RFR on the permeability of the blood-brain barrier was further supported in an experiment by Neilly and Lin [1986]. They showed that ethanol, infused into the femoral vein, reduced the RFR-induced (3150 MHz, 30 W/cm^2 rms for 15 min on the left hemisphere of the brain) increase in penetration of Evan's blue into the brain of rats. Ethanol attenuated the RFR-induced increase in brain temperature.

Several studies used horseradish peroxidase as an indicator of blood-brain barrier permeability. An increase in horseradish peroxidase in the brain after systemic administration could be due to an increase in pinocytosis of the epithelial cells in the capillary of the brain, in addition to or instead of an increase in the leakiness of the blood-brain barrier. Pinocytosis can actively transport the peroxidase from the general blood circulation into the brain. An increase in the concentration of horseradish peroxidase was found in the brain of the Chinese hamster after 2 h of irradiation to continuous-wave 2450-MHz RFR at 10 mW/cm^2 (SAR 2.5 W/kg) [Albert, 1977]. The increase was more concentrated in the thalamus, hypothalamus, medulla, and cerebellum, and less in the cerebral cortex and hippocampus [Albert and Kerns, 1981]. Increases in horseradish peroxidase permeability were also observed in the brains of rats and Chinese hamsters exposed for 2 h to continuous-wave 2800-MHz RFR at 10 mW/cm^2 (SAR 0.9 W/kg for the rat and 1.9 W/kg for the Chinese hamster). Fewer brain areas were observed with horseradish peroxidase at 1 h postexposure and complete recovery was seen at 2 h [Albert, 1979a]. Sutton and Carroll [1979] also reported an increase in permeability of horseradish peroxidase to the brain of the rat, when the brain temperature was raised to 40-45 ^{o}C by focal heating of the head with continuous-wave 2450-MHz RFR. In addition, cooling the body of the animals before exposure could counteract this effect of the radiation. These results again point to the conclusion that the hyperthermic effect of the RFR can disrupt the blood-brain barrier.

Oscar and Hawkins [1977] reported increased permeability of radioactive mannitol and inulin, and no significant change in dextran permeability into the brain of rats exposed for 20 min to continuous-wave or pulsed 1300-MHz RFR at a power density of 1 mW/cm^2 (SAR 0.4 W/kg). Effect of the pulsed radiation was more prominent. A 'power window' effect was also reported in this study. Preston et al. [1979] exposed rats to continuous-wave 2450-MHz RFR for 30 min at different power densities (0.1-30 mW/cm^2, SARs 0.02-6 W/kg) and observed no significant change in radioactive mannitol distribution in various regions of the brain. In that paper, they suggested that an increase in regional blood flow in the brain could explain the results of Oscar and Hawkins [1977]. In further experiments Preston and Prefontaine [1980] reported no significant change in the permeability of radioactive sucrose to the brain of rats exposed with the whole body to continuous-wave 2450-MHz RFR for 30 min at 1 or 10 mW/cm^2 (SARs 0.2 and 2.0 W/kg) or with the head for 25 min at different power densities. Gruenau et al. [1982] also reported no significant change on the penetration of ^{14}C-sucrose into the brain of rats after 30 min of exposure to pulsed (2 µs pulses, 500 pps) or continuous-wave 2800-MHz RFR of various intensities (1-15 mW/cm^2 for the pulsed radiation, 10 and 40 mW/cm^2 for the continuous-wave radiation). Ward et al. [1982] irradiated rats with 2450-MHz RFR for 30 min at different power densities (0-30 mW/cm^2, SAR 0-6 W/kg) and studied entry of ^{3}H-inulin and ^{14}C-sucrose into different areas of the brain. Ambient temperature of exposure was at either 22, 30, or 40 ^{o}C. They reported no significant increase in penetration of both compounds into the brain due to RFR exposure; however, they reported an increase in ^{14}C-sucrose entry into the hypothalamus when the ambient temperature of exposure was at 40 ^{o}C. The increase was suggested to be due to the hyperthermia induced in the animals under such exposure conditions. In a further study, Ward and Ali [1985] exposed rats to 1700-MHz continuous-wave or pulsed (0.5 µs pulses, 1000 pps) RFR for 30 min with the radiation concentrated at the head of the animal (SAR 0.1 W/kg). They reported no significant change in permeability into the brain of ^{3}H-inulin and ^{14}C-sucrose after the exposure.

Oscar et al. [1981] did observe increased blood flow in various regions of the rat brain after 5 to 60 min of exposure to pulsed 2800-MHz (2 µs pulses, 500 pps) RFR at 1 or 15 mW/cm^2 (SARs 0.2 and 3 W/kg). At 1 mW/cm^2, increased blood flow (measured at ~6 min after exposure) was observed in 16 of the 20 brain areas studied with the largest increase in the pineal gland, hypothalamus, and temporal cortex. After exposure to the radiation at 15 mW/cm^2, the largest increases in blood flow were detected in the pineal gland, inferior colliculus, medial geniculate nucleus, and temporal cortex (the last three areas are parts of the auditory system). It is interesting that patterns of changes involving different brain areas are reported in different studies [Albert and Kerns, 1981; Goldman et al., 1984; Oscar et al., 1981]. One wonders if this is due to the different patterns of energy distribution in the brain leading to different patterns of increases in local cerebral blood flow, since different exposure conditions were used in these experiments.

Williams et al. [1984a-d] carried out a series of experiments to study the effect of RFR exposure on blood-brain barrier permeability to hydrophilic molecules. Unrestrained, conscious rats were used in these studies. The effects of exposure to continuous-wave 2450-MHz RFR at 20 or 65 mW/cm^2 (SAR 4 or 13 W/kg) for 30, 90, or 180 min were compared with those of ambient heating (42 ^{o}C)-induced hyperthermia and urea infusion, on sodium fluorescein, horseradish peroxidase, and ^{14}C-sucrose permeability into different areas of the brain. In general, they found that hyperosmolar urea was the most effective and ambient heating was as effective as hyperthermic RFR in increasing the tracer concentrations in the brain. However, significant increase of plasma concentrations of sodium fluorescein and ^{14}C-sucrose were also observed in the heat- and RFR-exposed animals, which might result from a decrease in renal function due to hyperthermia. Increase in tracer concentrations in the brain could be due to the increase in plasma concentrations. The authors concluded that RFR did not significantly affect the penetration of the tracers into the brain (via the blood-brain barrier). In the case of horseradish peroxidase, a reduced uptake into the brain was actually observed. The authors speculated that there was a decrease in pinocytotic activity in cerebral micro-vessels after exposure for 30 to 90 min to the radiation at 65 mW/cm^2.

A series of experiments was carried out to study the effect of RFR on the passage of drugs into the central nervous system. Drug molecules that are less lipid soluble are less permeable through the blood-brain barrier. Thus, their actions are confined mainly to the peripheral nervous system after systemic administration. The actions of methylatropine, a peripheral cholinergic antagonist, methylnaltrexone, a peripheral opiate antagonist, and domperidone, a peripheral dopamine antagonist on RFR-exposed rats were studied by Quock et al. [1986a,b; 1987]. After 10 min of irradiation of mice to continuous-wave 2450-MHz RFR at 20 mW/cm^2 (SAR 53 W/kg), they observed antagonism of the apomorphine (a dopamine agonist)-induced stereotypic climbing behavior by domperidone, the analgesic effect of morphine (an opiate) by methylnaltrexone, and the central effects of oxotremorine and pilocarpine (both cholinergic agonists) by methylatropine. The behavioral and physiological responses studied are due to the action of the agonists in the central nervous system and are normally not blocked by the peripheral antagonists used in these studies. Since the enhanced antagonist effects of the peripheral drugs cannot be due to an increase in cerebral blood flow after exposure to the RFR, Quock et al. [1986a] speculated that the effect may be due to the breakdown of capillary endothelial tight-junction or an increase in pinocytosis in the blood-brain barrier.

Neubauer et al. [1990] studied the penetration of rhodamine-ferritin complex into the blood-brain barrier of the rat. The compound was administered systemically to the animals and then the animals were irradiated with pulsed 2450-MHz RFR (10 μs pulses, 100 pps) for 15, 30, 60 or 120 min at an average power density of 5 or 10 mW/cm^2 (SAR of 2 W/kg). Capillary endothelial cells from the cerebral cortex of the rats were isolated immediately after exposure, and the presence of rhodamine-ferritin complex in the cells was determined by the fluorescence technique. An approximately two fold increase in the complex was found in the cells of animals after 30 min or more of exposure to the 10 mW/cm^2 radiation. No significant effect was observed at 5 mW/cm^2. Furthermore, pretreating the animals before exposure with the microtubular function inhibitor colchicine blocked the effect of the RFR. These data indicate an increase in pinocytotic activity in the cells forming the blood-brain barrier. In a more recent study [Lange and Sedmak, 1991], using a similar exposure system, a dose- (power density) dependent increase in the entry of Japanese encephalitis virus into the brain and lethality was reported in mice after 10 min of RFR exposure (power densities 10-50 mW/cm^2, SARs 24-98 W/kg). The blood-brain barrier is a natural barrier against the penetration of this virus to the brain. The authors also speculated that the high-intensity RFR caused an increase in pinocytosis of the capillary endothelial cells in the central nervous system and the viruses were carried inside by this process.

It is apparent that in the majority of the studies a high intensity of RFR is required to alter the permeability of the blood-brain barrier. Change in brain or body temperature seems to be a necessary condition for the effect to occur. In addition, permeability alteration could be due to a passive change in 'leakiness' or an increase in pinocytosis in the blood-brain barrier.

ELECTROPHYSIOLOGICAL EFFECTS OF RADIOFREQUENCY RADIATION

Electrophysiology of Neurons

Wachtel et al. [1975] and Seaman and Wachtel [1978] described a series of experiments investigating the effect of RFR (1500 and 2400 MHz) on neurons from the isolated abdominal ganglion of the marine gastropod, Aphysia. Two types of cells generating regular action potential spikes or bursts were studied. A majority of cells (87%) showed a decrease in the rate of the spontaneous activity when they were irradiated with RFR. 'Temperature' controls were run and in certain neurons convective warming produced an opposite effect (increased rate of activity) to that produced by RFR (decreased activity). Chou and Guy [1978] exposed temperature-controlled samples of isolated frog sciatic nerves, cat saphenous nerve, and rabbit vagus nerve to 2450-MHz RFR. They reported no significant change in the characteristics of the compound action potentials in these nerve preparations during exposure to either continuous-wave (SARs 0.3-1500 W/kg) or pulsed (peak SARs 0.3-220 W/kg) radiation. No direct field stimulation of neural activity was observed.

Arber and Lin [1985] recorded from Helix aspersa neurons irradiated with continuous-wave 2450-MHz RFR (60 min at 12.9 W/kg) at different ambient temperatures. The irradiation induced a decrease in spontaneous firing at medium temperatures of 8 and 21 ^{o}C, but not at 28 ^{o}C. However, when the neurons were irradiated with noise-amplitude-modulated 2450-MHz RFR (20% AM, 2 Hz-20 kHz) at SARs of 6.8 and 14.4 W/kg, increased membrane resistance and spontaneous activity were observed.

Evoked Potentials

Several studies investigated the effects of RFR on evoked potentials in different brain areas. The evoked potential is the electrical activity in a specific location within the central nervous system responding to stimulation of the peripheral nervous system. Johnson and Guy [1972] recorded the evoked potential in the thalamus of cats in response to stimulation of the contralateral forepaw. The animals were exposed to continuous-wave 918-MHz RFR for 15 min at power densities of 1-40 mW/cm^2 at the head. A power density-dependent decrease in latency of some of the late components, but not the initial response of the thalamic evoked potential was observed. These data were interpreted that RFR affected the multisynaptic neural pathway, which relates neural information from the skin to the thalamus and is responsible for the late components of the evoked potential. Interestingly, warming the body of the animals decreased the latency of both the initial and late components of the evoked potential.

Taylor and Ashleman [1975] recorded spinal cord ventral root responses to electrical stimulation of the ipsilateral gastrocnemius nerve in cats, using a polyethylene suction electrode. The spinal cord was irradiated with continuous-wave 2450-MHz RFR at an incident power of 7.5 W. Decreases in latency and amplitude of the reflex response were observed during exposure (3 min) and responses returned to normal immediately after exposure. They also reported that raising the temperature of the spinal cord produced electrophysiological effects similar to those of RFR.

Electrophysiology of Auditory Effect of Pulsed RFR

Electrophysiological methods have also been used to study the pulsed RFR-induced auditory effects in animals. The effect was first systemically studied in humans by Frey [1961] and has been reviewed by Chou et al. [1982a] and Lin [1978]. Evoked potential responses were recorded in the eighth cranial nerve, medial geniculate nucleus, and the primary auditory cortex (three components of the auditory system) in cats exposed to pulsed 2450-MHz RFR. These evoked responses were eliminated after damaging the cochlea [Taylor and Ashleman, 1974]. Guy et al. [1975] studied the threshold of evoked responses in the medial geniculate nucleus in the cat in response to pulsed RFR while background noise (50-15000 Hz, 60-80 dB) was used to interfere with the response. They reported that background noise did not significantly affect the threshold to the RFR response, but caused a large increase in threshold to sound stimulus applied to the ear. The authors speculated that RFR interacts with the high frequency component of the auditory response system. In the study, evoked potentials in brain sites other than those of the auditory system were also recorded during pulsed RFR stimulation.

Chou et al. [1975] confirmed the peripheral site of the auditory effect generation. They recorded cochlear microphonics in the guinea pig inner ear during stimulation with 918-MHz pulsed RFR. The response was similar in characteristics to the cochlear microphonics generated by a click. These data were further supplemented by the finding that the middle-ear was not involved in the pulsed RFR-induced auditory responses, since destruction of the middle ear did not abolish the RFR-induced evoked potential in the brainstem [Chou and Galambos, 1979].

Experiments [Chou and Guy, 1979b] studying the threshold of RFR auditory effect in guinea pigs using the brainstem auditory evoked responses showed that the threshold for pulses with pulse width less than 30 μs was related to the incident energy per pulse, and for larger duration pulses it was related to the peak power. In another study Chou et al. [1985b] measured the intensity-response relationship of brainstem auditory evoked response in rats exposed to 2450-MHz pulsed RFR (10 pps) of different intensities and pulse widths (1-10 μs) in a circularly polarized waveguide. They also confirmed in the rat that the response is dependent on the energy per pulse and independent of the pulse width (up to 10 μs in this experiment).

Lebovitz and Seaman [1977a,b] recorded responses from single auditory neurons in the auditory nerve of the cat in response to 915-MHz pulsed RFR. Responses are similar to those elicited by acoustic stimuli. Seaman and Lebovitz [1987; 1989] also recorded in the cat the responses of single neurons in the cochlear nucleus, a relay nucleus in the auditory system, to pulsed 915-MHz RFR applied to the head of the animal. The threshold of response to RFR pulses was determined and found to be low (SAR response threshold determined at the midline of the brain stem, where the cochlear nucleus is located, was 11.1 mW/g/pulse corresponding to a specific absorption threshold of 0.6 μJ/g/pulse.)

Electroencephalographic Recording

Various experiments studied the effects of acute and chronic RFR exposures on electroencephalograph (EEG). Measurement of electrical activity from the brain using external electrodes provides a non-invasive means of studying brain activity. Electroencephalograph is the summation of neural activities in the brain and provides a gross indicator of brain functions. It is generated by cell activity in the cerebral cortex around the area of recording, but it is modulated by subcortical input, e.g., from the thalamus. Sophisticated techniques and methods are available in the recording and analysis of EEG that provide useful knowledge on brain functions [da Silva, 1991].

In the early studies on the effects of RFR on EEG, metal electrodes were used in recording that distorted the field and possibly led to artifactual results [Johnson and Guy, 1972]. Saline filled glass electrodes [Johnson and Guy, 1972] and carbon loaded Teflon electrodes [Chou and Guy, 1979a] were used in later experiments to record the electrical activity in the brain of animals during RFR exposure. The carbon loaded Teflon electrode has conductivity similar to tissue and, thus, minimizes field perturbation. It can be used for chronic EEG and evoked potential measurements in RFR studies.

Baranski and Edelwejn [1968] reported that acute pulsed RFR (20 mW/cm^2) had little effect on the EEG pattern of rabbits that were given phenobarbital; however, after chronic exposure (7 mW/cm^2, 200 h), desynchronization (arousal) was seen in the EEG after phenobarbital administration, whereas synchronization (sedation) was observed in the controls [Baranski and Edelwejn, 1974]. Goldstein and Sisko [1974] also reported periods of alternating EEG desynchronization and synchronization in rabbits anesthetized with pentobarbital and then subjected to 5 min of continuous-wave 9300-MHz RFR (0.7-2.8 mW/cm^2). Duration of desynchronization correlated with the power density of the irradiation. Servantie et al. [1975] reported that rats exposed for 10 days to 3000-MHz pulsed (1 μs pulses, 500-600 pps) RFR at 5 mW/cm^2 produced an EEG frequency in the occipital cortex (as revealed by spectral analysis) synchronous to the pulse frequency of the radiation. The effect persisted a few hours after the termination of exposure. The authors proposed that the pulsed RFR synchronized the firing pattern of cortical neurons.

Dumansky and Shandala [1974] reported in the rat and rabbit that changes in EEG rhythm occurred after chronic RFR exposure (120 days, 8 h/day) using a range of power densities. The authors interpreted their results as an initial increase in excitability of the brain after RFR exposure followed by inhibition (cortical synchronization and slow wave) after prolonged exposure. Shandala et al. [1979] exposed rabbits to 2375-MHz RFR (0.01-0.5 mW/cm^2) 7 h/day for 3 months. Metallic electrodes were implanted in various regions of the brain (both subcortical and cortical areas) for electrical recording during the exposure period and postexposure. After 1 month of exposure at 0.1 mW/cm^2, the authors observed in the sensory-motor and visual cortex an increase in alpha-rhythm, an EEG pattern indicative of relaxed and resting states of an animal. An increase in activity in the thalamus and hypothalamus was also observed later. Similar effects were also seen in animals exposed to the RFR at 0.05 mW/cm^2; however, rats exposed to a power density of 0.5 mW/cm^2 showed an increase in delta waves of high amplitude in the cerebral cortex after 2 weeks of exposure, suggesting a suppressive effect on EEG activity.

Bawin et al. [1973] exposed cats to 147-MHz RFR amplitude-modulated at 8 and 16 Hz at 1 mW/cm^2. They reported changes in both spontaneous and conditioned EEG patterns. Interestingly, the effects were not observed at lower or higher frequencies of modulation. Takashima et al. [1979] also studied the EEG patterns in rabbits exposed to RFR fields (1-30 MHz) amplitude-modulated at either 15 or 60 Hz. Acute exposure (2-3 h, field strength 60-500 V_{rms}/m) elicited no observable effect. Chronic exposure (2 h/day for 4-6 weeks at 90-500 V_{rms}/m) produced abnormal patterns including high amplitude spindles, bursts, and suppression of normal activity (shift to pattern of lower frequencies) when recorded within a few hours after exposure.

In an experiment by Chou and Guy [1979a], no significant change in electrical activity from the hypothalamus was detected in rabbits exposed to 2450-MHz RFR at 100 mW/cm^2 (SAR at electrode ~25 W/kg). In a chronic exposure experiment, Chou et al. [1982b] exposed rabbits to continuous-wave 2450-MHz RFR at 1.5 mW/cm^2 (2 h/day, 5 days/week for 90 days). Electroencephalograph and evoked potentials were measured at the sensory-motor and occipital cortex at various times during the exposure period. They reported large variations in the data and a tendency toward a decreased response amplitude in the latter part of the experiment, i.e., after a longer period of exposure.

In a more recent study, Chizhenkova [1988] recorded in the unanesthetized rabbits slow wave EEG in the motor and visual cortex, evoked potential in the visual cortex to light flashes, and single unit activity in the visual cortex during and after exposure to continuous-wave RFR (wavelength = 12.5 cm, 40 mW/cm^2, 1 min exposure to the head) using glass electrodes. She reported that RFR increased the incident of slow wave and spindles in the EEG, which are characteristics of slow wave sleep in animals. However, the radiation facilitated light-evoked responses in the visual cortex. Cells in the visual cortex also showed changes in firing rates (increase or decrease depending on the neuron studied). Driving responses of visual cortical neurons to light flashes, i.e., responses to sequence of light flashes of increasing frequency, were also enhanced by the RFR exposure. The author interpreted the data as showing a decrease in the threshold of visual evoked potential and an increase in excitability of visual cortical cells as a result of RFR exposure.

NEUROCHEMICAL EFFECTS OF RADIOFREQUENCY RADIATION

Neurochemical studies of RFR include those on the concentrations and functions of neurotransmitters, receptor properties, energy metabolism, and calcium efflux from brain tissues.

Changes in Neurotransmitter Functions

In most studies on the effects of RFR on neurotransmitter functions, only the concentration of neurotransmitters (usually measured as amount/gm wet weight of brain tissue) was measured in the brains of animals after irradiation. Data on change in concentration alone tells little about the nature of the effect, since it could result from different causes. For example, a decrease in the concentration could be due to an enhanced release or a decrease in synthesis of the neurotransmitter as the result of RFR exposure. For a more informative study, the turnover rate of a neurotransmitter should be investigated. This involves the measurement of the rate of decrease in concentration of the neurotransmitter when its synthesis is blocked and/or the rate of accumulation of the metabolites of the neurotransmitter. More recently, the rate of release of a neurotransmitter from a local brain region can be studied by the microdialysis technique.

Snyder [1971] reported a significant increase in the concentrations of serotonin and its metabolite, 5-hydroxyindolacetic acid, in the brain of rats after 1 h of exposure to continuous-wave 3000-MHz RFR at 40 mW/cm^2 (SAR 8 W/kg). However, decreases in both neurochemicals were observed in the brain of rats exposed 8 h/day for 7 days at 10 mW/cm^2. Thus, these results indicated an increase in the synthesis and turnover of brain serotonin after acute exposure and a decrease after prolonged exposure to RFR. Furthermore, warming the animals by placing them in an incubator heated at 34 ^{o}C had no significant effect on the turnover rate of serotonin in the brain.

Catravas et al. [1976] also reported an increase in diencephalon serotonin concentration and activity of tryptophan hydroxylase, the synthesis enzyme for serotonin, in the rat after 8 daily (8 h/day) exposures to RFR at 10 mW/cm^2. No significant changes in activity of monoamine oxidase, the degradation enzyme of serotonin, was observed in the brain of the irradiated rats.

Zeman et al. [1973] investigated the effects of exposure to pulsed 2860-MHz RFR on γ-amino-butyric acid (GABA) in the rat brain. No significant difference was observed in GABA concentration nor the activity of its synthesis enzyme, L-glutamate decarboxylase, in the brains of chronic (10 mW/cm^2, 8 h/day for 3-5 days, or 4 h/day, 5 days/week for 4 or 8 weeks) or acutely exposed (40 mW/cm^2 for 20 min, or 80 mW/cm^2 for 5 min) rats compared with those of the sham-exposed animals.

Rats exposed to continuous-wave 1600-MHz RFR at 30 mW/cm^2 for 10 min were reported to have altered concentrations of catecholamines (norepinephrine and dopamine) and serotonin in specific regions of the brain [Merritt et al., 1976]. Norepinephrine was decreased only in the hypothalamus, whereas decrease in serotonin was seen in the hippocampus and decreases in dopamine were observed in the striatum and hypothalamus. These effects were suggested to be caused by an uneven distribution of RFR in different regions of the brain. In a further study, rats exposed to similar radiation (20 or 80 mW/cm^2) were found to have a reduction of norepinephrine concentration in the basal hypothalamus, whereas no significant changes in dopamine and serotonin concentrations were observed even though the brain temperature increased up to 5 ^{o}C [Merritt et al., 1977]. In another study [Grin, 1974], rats were exposed to 2375-MHz RFR at power densities of 50 and 500 μW/cm^2 for 30 days (7 h/day). At 50 μW/cm^2, brain epinephrine was increased on the 20th day of exposure, but returned to normal by day 30. There were slight increases in norepinephrine and dopamine concentrations throughout the exposure period. At 500 μW/cm^2, concentrations of all three neurotransmitters were increased at day 5, but declined continually after further exposure.

Various studies have been carried out to investigate the neurochemical effects of RFR irradiation on acetylcholine in the brain. A decrease in whole brain concentration of acetylcholine, suggesting an increased release of the neurotransmitter, has been reported in mice exposed to a single 2450-MHz RFR pulse, which deposited 18.7 J in the brain and increased the brain temperature by 2 to 4 ^{o}C [Modak et al., 1981]. Several studies investigated the effect on acetylcholinesterase (AChE), the degradation enzyme for acetylcholine. Acute (30 min) exposure to 9700-MHz RFR was reported to inhibit the membrane-bound AChE activity in a vagal-heart preparation [Young, 1980]. This effect was attributed to a release of bound calcium from the postjunctional membrane. In another study [Baranski, 1972], acute exposure to pulsed RFR

(~3000 MHz) at 25 mW/cm^2 caused a decrease in AChE activity in the guinea pig brain. The effect was most pronounced at the diencephalon and mesencephalon (midbrain). After three months (3 h/day) of exposure at a power density of 3.5 mW/cm^2, an increase in brain AChE was observed. Surprisingly, when rabbits were subjected to the same chronic exposure treatment, a decrease in AChE activity was seen. On the other hand, two groups of investigators [Galvin et al., 1981; Miller et al., 1984] showed independently that 2450-MHz RFR exposure at a wide range of SARs did not significantly affect the activity of isolated AChE in vitro. More recently, Dutta et al. [1992] reported an increase in AChE activity in neuroblastoma cells in culture after 30 min of exposure to 147-MHz RFR amplitude-modulated at 16 Hz at SARs of 0.05 and 0.02 W/kg, but not at 0.005, 0.01, or 0.1 W/kg. The authors suggested a 'power window' effect. It is not known whether the effect was a response to the radiofrequency or the 16-Hz component of the radiation. Acetylcholinesterase is a very effective enzyme. A large decrease in its activity will be needed before any change in cholinergic functions can be observed.

D'Inzeo et al. [1988] reported an experiment that showed the direct action of RFR on acetylcholine-related ion channels in cultured chick embryo myotube cells. The acetylcholine-induced opening and closing of a single channel in the membrane of these cells were studied by the patch-clamp technique. Changes in membrane current of the whole cell in response to acetylcholine was also studied. The channels were probably the nicotinic cholinergic receptor channels, which are ligand-gated channels. The cell culture was exposed to continuous-wave 10750-MHz RFR with the power density at the cell surface estimated to be a few μW/cm^2. (Power density of the incident field at the surface of the culture medium was 50 μW/cm^2.) Recordings were made during exposure. The authors reported a decrease in acetylcholine-activated single channel opening, whereas the duration of channel opening and the conductance of the channels were not significantly affected by the radiation. Since these latter two parameters are temperature-dependent, the effect observed was suggested as not related to the thermal effects of RFR. The whole cell membrane current also showed an increase in the recovery rates (desensitization) during irradiation. Thus, RFR decreased the opening probability of the acetylcholine channel and increased the rate of desensitization of the acetylcholine receptors. Opening and desensitization of the nicotinic channels are known to involve different molecular mechanisms.

Lai et al. [1987b,c] performed experiments to investigate the effects of RFR exposure on the cholinergic systems in the brain of the rat. Activity of the two main cholinergic pathways, septo-hippocampal and basalis-cortical pathways, were studied. The former pathway has the cell bodies in the septum and their axons innervate the hippocampus. The latter pathway includes neurons in the nucleus basalis and innervates several cortical areas including the frontal cortex. These two cholinergic pathways are involved in many behavioral functions such as learning, memory, and arousal [Steriade and Biesold, 1990]. Degeneration of these pathways occurs in Alzheimers disease [Price et al., 1985]. In some studies, cholinergic activities in the striatum and hypothalamus were also investigated. Cholinergic activity in the brain tissue was monitored by measuring sodium-dependent high-affinity choline uptake (HACU) from brain tissues. Sodium-dependent high-affinity choline is the rate limiting step in the synthesis of acetylcholine and has widely been used as an index of cholinergic activity in neural tissue [Atweh et al., 1975].

We found that after 45 min of acute exposure to pulsed 2450-MHz RFR (2 μs pulses, 500 pps, 1 mW/cm^2, average whole body SAR 0.6 W/kg), HACU was decreased in the hippocampus and frontal cortex, whereas no significant effect was observed in the striatum, hypothalamus, and inferior colliculus [Lai et al., 1987b]. Interestingly, the effect of RFR on HACU in the hippocampus was blocked by pretreatment of the animals with the opiate-antagonists naloxone and naltrexone, suggesting involvement of endogenous opioids in the effect. Endogenous opioids are a group of peptides synthesized by the nervous system and have pharmacological properties like opiates. They are involved in a variety of physiological functions such as stress reactions, temperature-regulation, motivational behaviors, etc. Our further research showed that the effects of RFR on central cholinergic activity could be classically conditioned to cues in the exposure environment [Lai et al., 1987c]. These effects of RFR on cholinergic functions are similar to those reported in animals after exposure to stressors [Finkelstein et al., 1985; Lai, 1987; Lai et al., 1986c].

When different power densities of RFR were used, a dose-response relationship could be established from each brain region [Lai et al., 1989a]. Data were analyzed by probit analysis, which enables a statistical comparison of the dose-response functions of the different brain regions. It was found that a higher dose-rate was required to elicit a change in HACU in the striatum, whereas the responses of the frontal cortex and hippocampus were similar. Thus, under the same irradiation conditions, different brain regions could have different sensitivities to RFR.

In further experiments to investigate the contributory effect of different parameters of RFR exposure, we found that the radiation caused a duration-dependent biphasic effect on cholinergic activity in the brain. After 20 instead of 45 min of RFR exposure as in earlier experiments, an increase in HACU was observed in the frontal cortex, hippocampus, and hypothalamus of the rat [Lai et al., 1989b], and these effects could be blocked by pretreatment with the opiate antagonist naltrexone, suggesting the effects are also mediated by endogenous opioids.

Experiments [Lai et al., 1988] were then carried out to compare the effects of exposure in two different systems that produced different energy absorption patterns in the body of the exposed animal. Rats were exposed to pulsed (2 μs pulses, 500 pps) or continuous-wave 2450-MHz RFR in the circular waveguide and the miniature anechoic chamber exposure systems designed by Guy [Guy, 1979; Guy et al., 1979] with the whole body average SAR kept at a constant level of 0.6 W/kg. In the circular waveguide rats were exposed to circularly polarized RFR from the side of the body. In the miniature anechoic chamber rats were exposed dorsally with plane-polarized RFR. The circular waveguide produced a more localized energy absorption pattern than the miniature anechoic chamber. Detailed dosimetry studies in the body and brain of rats exposed in these two exposure systems had been carried out [Chou et al., 1984, 1985a]. After 45 min of exposure to the RFR, a decrease in HACU was observed in the frontal cortex in all exposure conditions studied (circular waveguide vs miniature anechoic chamber, pulsed vs continuous-wave). However, regardless of the exposure system used, HACU in the hippocampus decreased only after exposure to pulsed, but not continuous-wave RFR. Striatal HACU was decreased after exposure to either pulsed or continuous-wave RFR in the miniature anechoic chamber, but no significant effect was observed when the animal was exposed in the circular waveguide. No significant effect on HACU was found in the hypothalamus under all the exposure conditions studied. Thus, each brain region responded differently to RFR exposure depending on the parameters. Effects on the frontal cortex were independent of the exposure system or use of pulsed or continuous- wave RFR. The hippocampus only responded to pulsed but not to continuous-wave RFR. Response of the striatum depended on the exposure system used. The neurochemical changes were correlated with the dosimetry data of Chou et al. [1985a] on the local SARs in different brain areas of rats exposed to RFR in these two exposure systems. The dosimetry data showed that the septum, where the cell bodies of the hippocampal cholinergic pathway are located, had the lowest local SAR among eight brain areas measured in both exposure systems; however, the hippocampus cholinergic pathway responded to pulsed, but not to continuous-wave RFR. Dosimetry data from the frontal cortex showed a wide range of local SARs in the frontal cortex (0.11-1.85 W/kg per mW/cm^2) depending on the exposure system. Yet, exposure in both systems produced similar neurochemical responses in the frontal cortex (30-40% decrease in HACU). More interestingly, in the striatum the local SAR was approximately five times higher when the animals were exposed in the circular waveguide than in the miniature anechoic chamber; however, the striatal cholinergic system responded when the animal was exposed in the miniature anechoic chamber, but not in the circular waveguide. Since the cholinergic innervations in the striatum are mostly from interneurons inside the brain structure, these data would argue against a direct action of RFR on striatal cholinergic neurons causing a decrease in HACU, e.g., a local heating by the radiation. Unless different brain areas have different sensitivities to the direct effect of RFR, we could conclude that the effects of RFR on HACU in the brain areas studied in our experiments originated from other sites in the brain or body.

Neurotransmitter Receptors

Further experiments were conducted to investigate the effects of repeated RFR exposure on the cholinergic systems in the brain. Muscarinic cholinergic receptors were studied using the receptor-binding technique with ^{3}H-quinuclidinyl benzilate (QNB) as the ligand. These receptors are known to change their properties after repeated perturbation of the cholinergic system and that such changes can affect an animal's normal physiological functions [Overstreet and Yamamura, 1979]. After ten daily sessions of RFR exposure (2450 MHz at an average whole body SAR of 0.6 W/kg), the concentration of muscarinic cholinergic receptors changed in the brain [Lai et al., 1989b]. Moreover, the direction of change depended on the acute effect of the RFR. When animals were given daily sessions of 20-min exposure, which increased cholinergic activity in the brain, a decrease in the concentration of the receptors was observed in the frontal cortex and hippocampus. On the other hand, when animals were subjected to daily 45-min exposure sessions that decreased cholinergic activity in the brain, an increase in the concentration of muscarinic cholinergic receptors in the hippocampus resulted after repeated exposure and no significant effect was observed in the frontal cortex. These data pointed to an important conclusion that the long term biological consequence of repeated RFR-exposure depended on the parameters of exposure. Further experiments showed that changes in cholinergic receptors in the brain after repeated RFR exposure also depended on endogenous opioids, because the effects could be blocked by pretreatment before each session of daily exposure with the narcotic antagonist naltrexone [Lai et al., 1991]. Interestingly, changes in neurotransmitter receptor concentration also have been reported in animals after a single episode of exposure to RFR [Gandhi and Ross, 1987]. In the experiment rats were irradiated with 700-MHz RFR at 15 mW/cm^2 to produce a rise in body temperature of 2.5 ^{o}C (~10 min) and in some animals the temperature was allowed to return to normal (~50 min). Alpha-adrenergic and muscarinic cholinergic receptors were assayed in different regions of the brain using ^{3}H-clonidine and ^{3}H-QNB as ligands, respectively. No significant change in binding was observed for both receptors studied at the time when the body temperature reached a 2.5 ^{o}C increase. Decreases in ^{3}H-clonidine binding in the cerebral cortex, hypothalamus, striatum, and hypothalamus, and an increase in ^{3}H-QNB binding in the hypothalamus were observed when the brains were studied at the time the body temperature returned to the base line level. The authors speculated that the receptor changes were thermoregulatory responses to the hyperthermia. It is not uncommon that the concentration of neurotransmitter receptors in the brain changes after a single exposure to drug or perturbation, e.g., stress [Estevez et al., 1984; Mizukawa et al., 1989].

Data from the above experiments and those described in the previous section indicate that the parameters of irradiation are important determinants of the outcome of the biological effect. Different durations of acute exposure lead to different biological effects and, consequently, the effects of repeated exposure depends upon the duration of each exposure session. On the other hand, the waveform of the irradiation was an important factor. This was seen in the differential effects that occurred after exposure to pulsed vs continuous-wave RFR, plane vs circularly polarized waves, and the pattern of energy absorption in the body of the animal. These data raised the question whether the whole body SAR could be used as the sole factor in considering the biological effects of RFR. Other exposure factors also should be considered.

A series of experiments were carried out to investigate the neural mechanisms mediating the effects of low-level RFR on the cholinergic systems of the rat brain. Our experiments [Lai et al., 1987b, 1989b] showed that some of the neurological effects of RFR are mediated by endogenous opioids in the brain. Since there are three types of endogenous opioid receptors, μ, δ, and κ, in the brain [Mansour et al., 1987; Katoh et al., 1990], the types of opioid receptors mediating the effects of RFR were studied in a further experiment [Lai et al., 1992b]. We found that RFR-induced decrease in HACU in the hippocampus could be blocked by injection of specific μ, δ, and κ opioid-antagonists into the lateral cerebroventricle of rats before exposure to RFR (2450 MHz, 45 min at an average whole body SAR of 0.6 W/kg). Supporting the previous finding that the RFR-induced decrease in HACU in the frontal cortex was not mediated by endogenous opioids [Lai et al., 1987b], all types of opioid receptor antagonists tested were not effective in blocking the effect in the frontal cortex.

More recent research showed that the effects of RFR on both frontal cortical and hippocampal cholinergic systems could be blocked by pretreatment with an intracerebroventricular injection of the corticotropin-releasing factor (CRF) antagonist α–helical-CRF$_{9\text{-}41}$ [Lai et al., 1990]. Corticotropin-releasing factor is a hormone that has been implicated in mediating stress responses in animals [Fisher, 1989]. From the above results and data from our other research [Lai and Carino, 1990a], the following sequence of events in the brain was proposed [Lai, 1992] to be triggered by RFR:

cholinergic system

Radiofrequency radiation (2450-MHz, 45 min exposure at an average whole body SAR of 0.6 W/kg) activates CRF, which in turn caused a decrease in the activity of the cholinergic innervations in the frontal cortex and hippocampus of the rat. In addition, the effect of CRF on the hippocampal cholinergic system was mediated by endogenous opioids via μ, δ, and κ receptors. Since these effects can be blocked by direct injection of antagonists into the ventricle of the brain, the neural mechanisms involved are located inside the central nervous system.

A series of experiments were performed to study the effects of RFR on benzodiazepine receptors in the brain. Benzodiazepine receptors have been suggested to be involved in anxiety and stress responses in animals [Polc, 1988] and have been shown to change after acute or repeated exposure to various stressors [Braestrup et al., 1979; Medina et al., 1983a, b]. Exposure to RFR has been previously shown to affect the behavioral actions of benzodiazepines [Johnson et al., 1980; Thomas et al., 1979]. After an acute (45 min) exposure to 2450-MHz RFR (average whole body SAR 0.6 W/kg), increase in the concentration of benzodiazepine receptors occurred in the cerebral cortex of the rat, but no significant effect was observed in the hippocampus and cerebellum. Furthermore, the response of the cerebral cortex adapted after repeated RFR exposure (ten 45-min sessions) [Lai et al.,1992a].

Metabolism of Neural Tissues

With the changes in neurotransmitter functions after exposure to RFR, it would not be surprising to observe changes in second messenger activity in neural tissues that mediate the the reaction between a neurotransmitter and its receptors on the cell membrane. Studies in this area are sparse. Gandhi and Ross [1989] reported that exposure of rat cerebral cortex synaptosomes to 2800-MHz RFR at power densities greater than 10 mW/cm^2 (SAR, 1 mW/gm per mW/cm^2) increased ^{32}Pi incorporation into phosphoinositides, thereby suggesting an increase in inositol metabolism. These phospholipids play an important role in membrane functions and act as second messengers in the transmission of neural information between neurons.

Several studies have investigated the effects of RFR exposure on energy metabolism in the rat brain. Sanders and associates studied the components of the mitochrondrial electron-transport system that generates high energy molecules for cellular functions. The compounds nicotinamide adenosine dinucleotide (NAD), adenosine triphosphate (ATP), and creatine phosphate (CP) were measured in the cerebral cortex of rats exposed to RFR.

Sanders et al. [1980] exposed the head of rats to 591-MHz continuous-wave RFR at 5.0 or 13.8 mW/cm^2 for 0.5-5 min (local SAR at the cortex of the brain was estimated to be between 0.026 and 0.16 W/kg per mW/cm^2). Decreases in ATP and CP and an increase in NADH (the reduced form of NAD) concentration were observed in the cerebral cortex. These changes were found at both power densities of exposure. Furthermore, the authors reported no significant change in cerebral cortical temperature at these power densities. They concluded that the radiation decreased the activity of the mitochrondrial electron-transport system.

In another study [Sanders and Joines, 1984] the effects of hyperthermia and hyperthermia plus RFR were studied. The authors reported brain temperature-dependent decreases in ATP and CP concentrations in the brain. Radiofrequency radiation (591 MHz, continuous- wave, at 13.8 mW/cm^2, for 0.5-5 min) caused a further decline in the concentration of the compounds in addition to the temperature effect.

Sanders et al. [1984] further tested the effect of different frequencies of radiation (200, 591 and 2450 MHz) on the mitochrondrial electron-transport system. The effect on the concentration of NADH was found to be frequency dependent. An intensity-dependent increase in NADH level was observed in the cerebral cortex when irradiated with the 200-MHz and 591-MHz radiations. No significant effect was seen with the 2450-MHz radiation. In their paper, Sanders et al. [1984] made an interesting deduction. Under normal conditions, the concentration of ATP in a cell is maintained by conversion of CP into ATP by the enzyme creatine phosphate kinase. Thus, the concentration of ATP is generally more stable than that of CP, and the concentration of ATP does not decline unless the CP concentration has reached 60% of normal. In the case of the RFR, the concentration of ATP dropped as fast as the CP level. Thus, they speculated that the radiation may have inhibited creatine phosphate kinase activity in the brain tissue.

In a further study [Sanders et al., 1985], the effects of continuous-wave, sinusoidally amplitude-modulated, and pulsed 591-MHz RFR were compared after five min of exposure at power densities of 10 and 20 mW/cm^2 (SARs at the cerebral cortex were 1.8 and 3.6 W/kg). Different modulation frequencies (4-32 Hz) were used in the amplitude-modulation mode. There was no significant difference in the effect on the NADH level across the modulation frequency. Furthermore, pulsed radiations of 250 and 500 pps (5 μs pulses) were compared with power densities ranging from 0.5-13.8 mW/cm^2. The 500 pps radiation was found to be significantly more effective in increasing the concentration of NADH in the cerebral cortex than the 250 pps radiation. Since changes in these experiments occurred when the tissue (cerebral cortex) temperature was normal, the authors speculated that they were not due to hyperthermia, but to a direct inhibition of the electron-transport functions in the mitochrondria by RFR-induced dipole molecular oscillation in divalent metal containing enzymes or electron transport sites.

Another experiment related to brain metabolism after RFR exposure was performed by Wilson et al. [1980]. They studied the uptake of ^{14}C-2-deoxy-D-glucose (2-DG) in the auditory system of the rat after exposure to either pulsed 2450 MHz (20 μs pulses, 10 pps, average power density 2.5 mW/cm^2) or continuous-wave 918-MHz (2.5-10 mW/cm^2) RFR for 45 min. One middle ear of the rats was destroyed before the experiment. Neurons that have increased activity (metabolism) will pick up an increased amount of 2-DG, which will accumulate in the cell body, since it is not a normal substrate for cellular functions. Location in the brain of these neurons can then be identified histologically by the autoradiographic technique. The authors reported a symmetrical (in both brain hemispheres) increase in 2-DG uptake in the inferior colliculus, medial geniculate nucleus, and various other nuclei in the auditory system after exposure. Asymmetric (contralateral to the intact middle ear) uptake was seen in the auditory system of rats exposed to auditory stimuli. Further experiment showed that unilateral destruction of the cochlea before the experiment produced asymmetric 2-DG uptake in the brain after exposure to the RFR. These data confirmed the findings of Chou et al. [1975] and Chou and Galambos [1979] that the cochlea and not the middle ear contributes to the auditory perception of pulsed RFR. However, it is surprising that both continuous-wave and pulsed RFRs produced similar patterns of 2-DG uptake in the auditory system and only pulsed RFR elicited auditory sensation.

Calcium Efflux

Another important topic of research on the neurochemical effects of electromagnetic radiation is the efflux of calcium ions from brain tissue. Calcium ions play important roles in the functions of the nervous system, such as the release of neurotransmitters and the actions of some neurotransmitter receptors. Thus, changes in calcium ion concentration could lead to alterations in neural functions.

Bawin et al. [1975] reported an increase in efflux of calcium ions from chick brain tissue after 20 min of exposure to a 147-MHz RFR (1 to 2 mW/cm^2). The effect occurred when the radiation was sinusoidally amplitude-modulated at 6, 9, 11, 16, or 20 Hz, but not at modulation frequencies of 0, 0.5, 3, 25, or 35 Hz. The effect was later also observed with 450-MHz radiation amplitude-modulated at 16 Hz, at a power density of 0.75 mW/cm^2. Bicarbonate and pH of the medium were found to be important factors in the effect [Bawin et al., 1978].

In vitro increase in calcium efflux from the chick brain was further confirmed by Blackman et al. [1979, 1985, 1980a,b] using amplitude-modulated 147-MHz and 50-MHz RFR. They also reported both modulation-frequency windows and power windows in the effect. These data would argue against a role of temperature. The existence of a power-density window on calcium efflux was also reported by Sheppard et al. [1979] using a 16-Hz amplitude-modulated 450-MHz field. An increase in calcium ion efflux was observed in the chick brain irradiated at 0.1 and 1.0 mW/cm^2, but not at 0.05, 2.0, or 5.0 mW/cm^2.

Two other papers reported no significant change in calcium efflux from the rat brain irradiated with RFR. Shelton and Merritt [1981] exposed rat brains to 1000-MHz RFR pulse-modulated with square waves (16 and 32 Hz, power density 0.5-15 mW/cm^2). They observed no change in calcium efflux from the tissue. Merritt et al. [1982] exposed rat brains with either 1000-MHz pulsed radiation modulated at 16 Hz at 1 or 10 mW/cm^2 (SARs 0.29 and 2.9 W/kg), or to a pulse-modulated 2450-MHz RFR at 1 mW/cm^2 (SAR 0.3 W/kg). No significant change in calcium efflux was observed in this experiment. These researchers also exposed animals, in vivo, injected with radioactive calcium to pulsed 2060-MHz RFR at different combinations of intensities and pulse repetition rates. No significant change in radioactive calcium content was found in the brains of the animals after 20 min of exposure. It is not known whether the discrepancies between these data and the findings of Bawin et al. [1975, 1978] and Blackman et al. [1979] were due to the use of square-wave instead of sinusoidally modulated radiation or due to the different species of animals studied. Electromagnetic field-induced increases in calcium efflux have also been reported in tissues obtained from different species of animals. Adey et al. [1982] observed an increase in calcium efflux from the brain of conscious cats paralyzed with gallamine and exposed for 60 min to a 450-MHz field (amplitude modulated at 16 Hz at 3.0 mW/cm^2, SAR 0.20 W/kg). Lin-Liu and Adey [1982] also reported increased calcium efflux from synaptosomes prepared from the rat cerebral cortex when irradiated with a 450-MHz RFR amplitude-modulated at various frequencies (0.16-60 Hz). Again, modulation at 16 Hz was found to be the most effective. More recently, Dutta et al. [1984] reported radiation-induced increases in calcium efflux from cultured cells of neural origins. Increases were found in human neuroblastoma cells irradiated with 915-MHz RFR (SARs 0.01-5.0 W/kg) amplitude-modulated at different frequencies (3-30 Hz). A modulation frequency window was reported. Interestingly, at certain power densities, an increase in calcium efflux was also seen with unmodulated radiation. A later paper [Dutta et al., 1989] reported increased calcium efflux from human neuroblastoma cells exposed to 147-MHz RFR amplitude-modulated at 16 Hz. A power window (SAR between 0.05-0.005 W/kg) was observed. When the radiation at 0.05 W/kg was studied, peak effects were observed at modulation frequencies between 13-16 Hz and 57.5-60 Hz. In addition, the authors also reported increased calcium efflux in another cell line, the Chinese hamster-mouse hybrid neuroblastoma cells. Effect was observed when these cells were irradiated with a 147-MHz radiation amplitude-modulated at 16 Hz (SAR 0.05 W/kg).

In more recent studies, Blackman explored the effects of different exposure conditions [Blackman et al., 1988, 1989, 1991]. Multiple power windows of calcium efflux from chick brains were reported. Within the power densities studied in this experiment (0.75-14.7 mW/cm^2, SAR 0.36 mW/kg per mW/cm^2) narrow ranges of power density with positive effect were separated by gaps of no significant effect. The temperature in which the experiment was run was also reported to be an important factor of the efflux effect. A hypothetical model involving the dynamic properties of cell membrane has been proposed to account for these effects [Blackman et al., 1989].

In addition to calcium ion, changes in other trace metal ions in the central nervous system have also been reported after RFR exposure. Stavinoha et al. [1976] reported an increase in zinc concentration in the cerebral cortex of rats exposed to 19-MHz RFR. Increases in the concentration of iron in the cerebral cortex, hippocampus, striatum, hypothalamus, midbrain, medulla, and cerebellum; manganese in the cerebral cortex and medulla; and copper in the cerebral cortex were reported in the rat after 10 min of exposure to 1600-MHz RFR at 80 mW/cm^2 (SAR 48 W/kg) [Chamness et al., 1976]. The significance of these changes is not known. The effects could be as a result of hyperthermia, because the colonic temperature of the animals increased by as much as 4.5 ^oC after exposure.

RADIOFREQUENCY RADIATION AND THE ACTIONS OF PSYCHOACTIVE DRUGS

The actions of psychoactive drugs depend on the functions of the neurotransmitter systems in the brain. Changes in neurotransmitter functions after RFR exposure will inevitably lead to changes in the actions of psychoactive drugs administered to the animal. On the other hand, if there is no change in the pharmacokinetics of drugs after RFR exposure, observed changes in psychoactive drug actions would imply RFR-induced changes in neurotransmitter functions in the animal. Pharmacological studies of RFR effects provide an important insight into the neural mechanisms affected by exposure to RFR.

Psychoactive drugs of various types have been tested in animals after exposure to RFR. Since an effect of RFR is to increase the body temperature of an animal, special attention has been given to study the effects of psychoactive drugs on the thermal effect of RFR. Jauchem [1985] has reviewed the effects of drugs on thermal responses to RFR. Radiofrequency radiation of high power densities was used in these studies.

Some psychoactive drugs have a profound effect on thermoregulation and, thus, alter the body temperature of an animal upon administration. The effect could be due to direct drug action on the thermoregulatory mechanism within the central nervous system or effects on autonomic functions such as respiration, cardiovascular and muscular systems, which lead to changes in body temperature. Several studies have investigated the neuroleptic (anti-psychotic) drug, chlorpromazine. Michaelson et al. [1961] reported that chlorpromazine enhanced the thermal effect of RFR in dogs (2800 MHz, pulsed, 165 mW/cm^2). Drug-treated animals had a faster rate of body temperature increase and a higher peak temperature when irradiated with RFR. Similar effects were seen with pentobarbital and morphine sulfate. On the other hand, Jauchem et al. [1983, 1985] reported that chlorpromazine attenuated the thermal effect of RFR in ketamine anesthetized rats. The drug slowed the rate of rise in colonic temperature (from 38.5-39.5 ^oC) and facilitated the return to base line temperature after exposure to RFR (2800-MHz, 14

W/kg); however, when the body temperature was allowed to rise to a lethal level, chlorpromazine potentiated the effect of RFR. Interestingly, haloperidol, another neuroleptic drug, was found to have no significant effect on RFR-induced change in colonic temperature. In another study [Lobanova, 1974b], the hyperthermic effect of RFR (40 mW/cm^2) was found to be attenuated by pretreatment with chlorpromazine or acetylcholine and enhanced by epinephrine and atropine (a cholinergic antagonist). This suggests a role of acetylcholine in modifying RFR-induced hyperthermia. Indeed, Ashani et al. [1980] reported that acute RFR exposure (10 min at 10 mW/cm^2) enhanced the hypothermic effects of AChE inhibitors. On the other hand, Jauchem et al. [1983, 1984] observed no significant effect of atropine and propranolol (an adrenergic antagonist) on the hyperthermia produced in ketamine anesthesized rats exposed to 2800-MHz RFR (SAR 14 W/kg).

Several studies investigated the effects of RFR on the actions of barbituates. Barbituates are sedative-hypnotic compounds, which produce narcosis (sleep states and loss of consciousness), synchronization of EEG, and poikilothermia (i.e., loss of body temperature regulatory functions). Baranski and Edelwejn [1974] reported that acute exposure to pulsed RFR (20 mW/cm^2) had little effect on the EEG pattern of rabbits given phenobarbital; however, after 200 h of exposure (at 7 mW/cm^2), desynchronization rather than synchronization of the EEG pattern was seen after phenobarbital administration. Rabbits anesthetized with pentobarbital and subjected to 5 min of RFR (0.7-2.8 mW/cm^2) showed periods of alternating EEG arousal (desynchronization) and sedation (synchronization) and periods of behavioral arousal. The duration of EEG arousal seemed to correlate with the power density of RFR [Goldstein and Sisko, 1974].

Wangemann and Cleary [1976] reported that short term RFR exposure (5-50 mW/cm^2) decreased the duration of pentobarbital induced loss of righting reflex in the rabbit. The investigators speculated that the effect was due to the thermal effect of RFR, which decreased the concentration of pentobarbital in the central nervous system. Supporting this, Bruce-Wolfe and Justesen [1985] reported that warming an animal with RFR while under anesthesia could attenuate the effects of pentobarbital. Mice exposed to continuous-wave 2450-MHz RFR at 25 and 50 mW/cm^2 also showed a power density-dependent reduction in the duration of hexobarbital-induced anesthesia [Blackwell, 1980]. On the other hand, Benson et al. [1983] reported decreased onset-time and prolonged duration of phenobarbital-induced narcosis in mice after exposure to RFR (10 mW/cm^2, 10 min). They showed that the effect was caused by an increase in deposition of phenobarbital in the brain. We [Lai et al., 1984a] have shown that after 45 min of exposure to pulsed 2450-MHz RFR (2 µs pulses, 500 pps, whole-body average SAR 0.6 W/kg), the pentobarbital-induced narcosis and hypothermia in the rat were enhanced. We also found that exposure of rats in two different orientations (with the head of the rat facing or away from the source of the RFR) had different effects on the pentobarbital-induced hypothermia, even though the average whole body SAR was similar under the two conditions. These data suggest that the pattern of localized SAR in the body of the animal might be an important determinant of the outcome of the effect.

When the body temperature of an animal is raised above a certain level, convulsions result. Various psychoactive drugs were studied in an attempt to alter the convulsive effect of RFR. Studies have also been carried out to investigate whether RFR exposure altered the potency of convulsants. It was reported that the susceptibility of rats to the convulsive effect of RFR (14 mW/cm^2, 2 h) was decreased by chloral hydrate, sodium pentobarbital, and bemegride, and enhanced by chlorpromazine, epinephrine, atropine, acetylcholine, nicotine, and monoamine oxidase inhibitors, but was not significantly affected by serotonin [Lobanova, 1974a]. Some of these results can be explained by the pharmacological properties of the drug tested. Pentobarbital and chloral hydrate are hypnotic agents and are known to have anticonvulsant effects. Chlorpromazine, nicotine, and monoamine oxidase inhibitors can lower the seizure threshold or induce convulsions depending on their dosages. Atropine, a cholinergic antagonist, has been shown to enhance the seizure threshold. It is puzzling that bemegride decreased RFR induced seizures, since it is a nervous system stimulant with similar pharmacological actions as the convulsant pentylenetetrazol.

Exposure to pulsed RFR (7 and 20 mW/cm^2) was reported to affect the effects of the convulsants, pentylenetetrazol and strychnine, on EEG activity [Baranski and Edelwejn, 1974]. Another study showed that low-level RFR altered the sensitivity of animals to the seizure inducing effect of pentylenetetrazol [Servantie et al., 1974]. Rats and mice were subjected to 8-36 days of pulsed RFR (3000 MHz, 0.9-1.2 μs pulses, 525 pps, 5 mW/cm^2). No significant change in susceptibility to the drug was seen after eight days of exposure; however, a decrease in susceptibility was observed after 15 days, and an increase in susceptibility was observed after 20, 27, and 36 days of irradiation. Mice became more susceptible to the convulsive effect of pentylenetetrazol and more animals died from convulsions. Thus, the sensitivity of the nervous system to the convulsive action of the drug changed as a function of the duration of exposure. In another study, Pappas et al. [1983] showed in the rat that acute (30 min) exposure to 2700-MHz pulsed RFR at 5, 10, 15, and 20 mW/cm^2 (SARs 0.75, 1.5, 2.25, and 3.0 W/kg, respectively) produced no significant interaction effect on pentylenetetrazol induced seizure or the efficacy of chlordiazepoxide (an anticonvulsant) to block the seizure.

Drugs affecting cholinergic functions in the nervous system have also been studied. Chronic RFR-exposed rats (10-15 days) were found to be less susceptible to the paralytic effect of curare-like drugs, which block nicotinic cholinergic transmission. A similar effect was observed on muscle preparations from the irradiated rats. Presumably, the cholinergic transmission in the neuromuscular junction was affected by RFR. Ashani et al. [1980] reported that acute pulsed RFR (10 min, 10 mW/cm^2) enhanced the hypothermic effects of an inhibitor of AChE (the degradation enzyme of acetylcholine). The site of this effect was determined to be located inside the central nervous system. Monahan [1988] also reported that RFR (2450 MHz, continuous-wave, whole body SARs 0.5-2.0 W/kg) affected the actions of scopolamine, a cholinergic antagonist, and physostigmine, a cholinergic agonist, on motor activity of mice in a maze. The data suggested enhancement of cholinergic activity after RFR irradiation.

Several studies investigated the actions of benzodiazepines, a group of drugs used for anticonvulsion, sedation-hypnosis, and antianxiety purposes. Two of the most commonly used benzodiazepines for the treatment of anxiety disorders are chlordiazepoxide (Librium) and diazepam (Valium). Low-level pulsed RFR (1 mW/cm^2, whole body SAR 0.2 W/kg) potentiated the effect of chlordiazepoxide on bar-pressing behavior of rats working on a DRL-schedule for food reinforcement; however, the same authors also reported no interaction effects between RFR and diazepam on bar pressing [Thomas et al., 1979, 1980].

Increase in brain benzodiazepine receptors in the brain after RFR exposure [Lai et al, 1992a] could explain the former effect. A possible explanation for the discrepancy of the results observed with chlordiazepoxide and diazepam was that diazepam has a higher potency than chlordiazepoxide. The potency of diazepam that was effective in attenuation of experimental conflict, an animal model of anxiety, was about four times that of chlordiazepoxide [Lippa et al., 1978], and the in vitro relative affinity of diazepam with benzodiazepine receptors was 30-65 times that of chlordiazepoxide [Braestrup and Squires, 1978; Mohler and Okada, 1977]. The ranges of diazepam and chlordiazepoxide used in the Thomas studies [Thomas et al., 1979, 1980] were 0.5-20 and 1-40 mg/kg, respectively. Thus, the doses of diazepam studied might be equivalent or higher in potency than the highest dose of chlordiazepoxide used. This supposition was supported by the observation in the Thomas studies that the effects of the two drugs were different. The dose-response curve of chlordiazepoxide on the DRL-schedule operant responses showed a dose-dependent inverted-U function, i.e., potentiation at medium dose, attenuation at higher dose, and only the portion of the response-curve that showed potentiation was affected by RFR [Thomas et al., 1979]. In the study of Thomas et al. [1980] on diazepam, only attenuation of DRL-responses was observed. Thus, the dose range of diazepam used in the study was at the attenuation portion of the dose-response function, which is not affected by RFR. These dose-dependent potentiation and attenuation effects of benzodiazepines on the operant response may involve different neural mechanisms. Radiofrequency radiation may only affect and enhance the potentiating and not the attenuating effect of benzodiazepines, which is possible because our research [Lai et al., 1992a] showed that the effect of RFR on benzodiazepine receptors is brain-region selective. Thus, the data of Thomas et al. [1979, 1980] on the interaction of RFR irradiation on benzodiazepine actions could be explained by a selective increase in benzodiazepine receptors in different regions of the brain. Another possibility is that RFR affects only the subtype of benzodiazepine receptors related to antianxiety effect and not another subtype related to the sedative-hypnotic action of the drugs. In the dose-response curve of benzodiazepine on DRL-schedule maintained behavior, the potentiation portion may be due to the former receptor subtypes and the attenuation portion the latter subtype. There is ample evidence suggesting that different subtypes of benzodiazepine receptors subserve antianxiety and sedative effects [Polc, 1988].

In addition to the above studies on the effect of RFR on benzodiazepines, Monahan and Henton [1979] trained mice to avoid or escape from 2450-MHz RFR (45 W/kg) under an avoidance paradigm. They reported that pretreatment of the animals with chlordiazepoxide decreased the avoidance response and increased the escape responses, which led to an increase in the animal's cumulative exposure to RFR after the drug treatment. The authors speculated that RFR potentiated the effect of chlordiazepoxide and caused a decrement in the avoidance response. It is also interesting that in the procedure the presence of RFR was signalled simultaneously with a tone and the animal could elicit an avoidance response, which resets the timer and delays the further presentation of RFR. Thus, the procedure had both signalled and continuous avoidance components. However, the data indicate that the effect was more like a continuous avoidance paradigm. Generally, anxiolyltic agents like benzodiazepines decrease both avoidance and escape behavior in a signalled-avoidance paradigm, but they can selectively decrease the avoidance response and leave the escape responding intact under a continuous avoidance paradigm.

Johnson et al. [1980] reported that repeated exposure (twenty-one 45-min sessions) to RFR (2450 MHz, pulsed, average whole body SAR 0.6 W/kg) reduced the sedative hypnotic effect, but increased the feeding behavior induced by diazepam. Hjeresen et al. [1987] reported that the attenuation effect of a single (45 min) RFR exposure (2450 MHz, CW, average whole body SAR 0.3 W/kg) on ethanol-induced hypothermia was blocked by treating the rat with the benzodiazepine antagonist, RO 15-1778. The data indicated that benzodiazepine receptors in the brain might mediate the effects of RFR on ethanol-hypothermia. In a more recent study, Quock et al. [1990] investigated the influence of RFR exposure on the effect of chlordiazepoxide on the stair-case test for mouse, a test for both the sedative and antianxiety effects of benzodiazepines. They reported that acute exposure (5 min at a whole body average SAR of 36 W/kg) caused a significant reduction of the sedative, but not the antianxiety effect of chlordiazepoxide. The effect was probably related to hyperthermia. Some of the above effects of RFR on benzodiazepine actions can be explained by our finding [Lai et al., 1992a] that acute RFR exposure increased benzodiazepine receptors in selective regions of the brain and that adaptation occurred after repeated exposure.

On the other hand, central benzodiazepine receptors can also affect seizure susceptibility in animals. Benzodiazepines are widely used as anticonvulsants. Exposure to RFR has been shown to affect seizure and convulsion susceptibility in animals. For example, Stverak et al. [1974] reported that chronic exposure to pulsed RFR attenuated audiogenic seizures in seizure-sensitive rats. Servantie et al. [1974] showed that mice chronically exposed to pulsed RFR initially showed a decrease and then an increase in susceptibility to the convulsant pentylenetetrazol. However, Pappas et al. [1983] showed no significant interaction effect of RFR on pentylenetetrazol-induced seizures nor the efficacy of chlordiazepoxide to block the seizure in rats. A more thorough study of the different parameters of RFR exposure on benzodiazepine receptors in the brain may explain these findings. Benzodiazepine receptors are very dynamic and can undergo rapid changes in properties in response to environmental stimuli [Braestrup et al., 1979; Lai and Carino, 1990b; Medina et al., 1983a,b; Soubrie et al., 1980; Weizman et al., 1989]. However, the direction of change and extent of effect depend on the stimulus and experimental conditions.

We conducted experiments to study the effect of acute RFR exposure on the actions of various psychoactive drugs [Lai et al., 1983; 1984a,b]. We found that acute (45 min) exposure to pulsed 2450-MHz RFR (2 μs pulses, 500 pps, 1 mW/cm^2, whole body average SAR 0.6 W/kg) enhanced apomorphine-hypothermia and stereotypy, morphine-catalepsy, and pentobarbital-hypothermia and narcosis, but it attenuated amphetamine-hyperthermia and ethanol-hypothermia. These psychoactive drugs are lipid-soluble and readily enter the central nervous system and the effects observed are not unidirectional, i.e., depending on the drug studied, increase or decrease in action was observed after RFR exposure. Therefore, these effects cannot be explained as a change in entry of the drugs into the brain, e.g., change in blood-brain barrier permeability or alteration in drug metabolism as a result of RFR exposure. Our finding that acute low-level RFR attenuated ethanol-hypothermia in the rat was replicated by Hjeresen et al. [1988] at a lower whole body average SAR of 0.3 W/kg. Blood ethanol level measurements indicated that the effect was not due to changes in metabolism or disposition of ethanol in the body. Results from further experiments [Hjeresen et al., 1989] suggested that the β-adrenergic mechanism in the brain might be involved in the attenuation effect of RFR on ethanol-induced hypothermia in the rat.

We further found that the effects of RFR on amphetamine-hyperthermia [Lai et al., 1986b] and ethanol-hypothermia could be classically conditioned to cues in the exposure environment after repeated exposure. Another interesting finding in our research was that some of the effects of RFR on the actions of the psychoactive drugs could be blocked by pretreating the rats with narcotic antagonists before exposure, suggesting the involvement of endogenous opioids [Lai et al., 1986b]. The hypothesis that low-level RFR activates endogenous opioids in the brain was further supported by an experiment showing that the withdrawal syndromes in morphine-dependent rats could be attenuated by RFR exposure [Lai et al., 1986a]. This hypothesis can explain most of the RFR-psychoactive drug interaction effects reported in our studies [see Table I in Lai et al., 1987a].

In another study [Lai et al., 1984b], water-deprived rats were allowed to drink a 10% sucrose solution from a bottle in the waveguide. Exposure to pulsed 2450-MHz RFR (2 μs pulses, 500 pps, 1 mW/cm^2, SAR 0.6 W/kg) did not significantly affect the consumption of the sucrose solution. However, when the sucrose solution was substituted by a 10% sucrose-15% ethanol solution, the rats drank ~25% more when they were exposed to the RFR than when they were sham exposed. The hypothesis that RFR activates endogenous opioids in the brain can also explain the increased ethanol consumption during RFR exposure. Recent studies have shown that activation of opioid mechanisms in the central nervous system can induce voluntary ethanol drinking in the rat [Nichols et al., 1991; Reid et al., 1991; Wild and Reid, 1990].

Frey and Wesler [1983] studied the effect of low-level RFR (1200 MHz, pulsed, 0.2 mW/cm^2, 15 min) on central dopaminergic functions. Radiofrequency radiation was found to attenuate the effect to both a high dose (1 mg/kg, IP) and a low dose (0.1 mg/kg, IP) of apomorphine on the latency of the tail-flick responses in the rat. The tail-flick test is a measure of pain perception in animals. These data are difficult to explain, since high dose and low dose of apomorphine affect predominantly the post- and presynaptic-dopamine receptors, respectively. These two types of dopamine receptors have opposite effects on dopamine transmission and functions. Other experiments indicating an effect of RFR on dopamine function in the brain are those of Michaelson et al. [1961] and Jauchem et al. [1983, 1985] showing the effect of chlorpromazine on RFR-induced hyperthermia, and our experiment showing an enhancement of apomorphine-hypothermia by RFR [Lai et al., 1983]. Chlorpromazine and apomorphine are dopamine antagonist and agonist, respectively. On the other hand, Thomas et al. [1980] reported no significant interaction effect between chlorpromazine and pulsed RFR (2800 MHz, 2 μs pulses, 500 pps, 1 mW/cm^2, SAR 0.2 W/kg) on rats responding on a fixed interval reinforcement schedule for food reward. However, Thomas and Maitland [1979] reported that exposure to pulsed 2450-MHz RFR (2 μs pulses, 500 pps, 1 mW/cm^2, SAR 0.2 W/kg) potentiated the effect of d-amphetamine on rats responding on a DRL-schedule of reinforcement. Amphetamine is an agonist of both dopamine and norepinephrine functions in the brain.

Two studies imply RFR affects serotonergic activity in the brain. Galloway and Waxler [1977] reported interaction between RFR and a serotonergic drug. Rhesus monkeys trained on a color-matching task were irradiated with continuous-wave 2450-MHz RFR at different dose rates. The animals were also treated with the serotonergic drug fenfluramine, which inhibits granule reuptake and storage of serotonin in nerve terminals and causes a long-lasting depletion of serotonin in the brain. Radiofrequency radiation alone had no significant effect on performance, whereas fenfluramine alone decreased the response accuracy and response rate in performing the task. Exposure to RFR plus the drug treatment produced a synergistic effect. A severe disruption of responding was observed. The authors speculated that RFR may act like fenfluramine, i.e., decreases serotonergic functions in the brain. This may be related to the finding of Frey [1977] who reported that RFR exposure decreased tail pinch- induced aggressive behavior in the rat. Fenfluramine and other drug treatments that decrease serotonergic functions in the brain were shown to suppress aggressive behavior elicited by electric foot-shock in rats [Panksepp et al., 1973].

Results from one of our experiments also indicated an increase in serotonergic activity in the brain of rats exposed to RFR. We [Lai et al., 1984c] observed an increase in body temperature (~1.0 ^{o}C) in the rat after acute (45 min) exposure to pulsed 2450-MHz RFR (2 μs pulses, 500 pps, 1 mW/cm^2, SAR 0.6 W/kg). This hyperthermic effect was blocked by pretreating the rats before exposure with the serotonin antagonists, cinanserin, cyproheptadine, and metergoline, but not by the peripheral serotonin antagonist, xylamidine, implying that the effect is mediated by serotonergic mechanism inside the central nervous system.

The findings that RFR can affect (potentiate or attenuate) the actions of psychoactive drugs could have important implication in considering the possible hazardous effects of the radiation. Most of the drugs studied, such as the benzodiazepines and neuroleptics, are widely used for therapeutic purposes. On the other hand, drugs can enhance the biological effects of RFR. Example are the studies of Kues and Monahan [1992] and Kues et al. [1990; 1992] showing synergistic effects of drugs on corneal endothelium damages and retinal degeneration in the monkey induced by repeated exposure to RFR. They found that application of the drugs timolol and pilocarpine to the eye before RFR exposure could lower the threshold of the RFR effect by 10 folds (from 10 to 1 mW/cm^2). Timolol and pilocarpine are commonly used in the treatment of glaucoma.

PSYCHOLOGICAL EFFECTS OF RADIOFREQUENCY RADIATION

A necessary consequence of change in neurological activity is a change in behavior. If RFR alters electrophysiological and neurochemical functions of the nervous system, changes in behavior will result. Effects of RFR on both spontaneous and learned behaviors have been investigated.

Spontaneous Behaviors

The effects of RFR on motor activity were the subjects of various studies. Changes in motor activity are generally regarded as indications of changes in the arousal state of an animal. Hunt et al. [1975] reported increased motor activity in rats after 30 min of exposure to 2450-MHz RFR (SAR of 6.3 W/kg) and decreased swimming speed in cold (24 ^{o}C) water. However, Roberti [1975] reported no significant change in locomotor activity in rats after long term (185-408 h) exposure to RFR at different frequencies and intensities (SARs 0.15-83 W/kg). Modak et al. [1981] reported a decrease in motor activity in rats exposed to a single pulse (15 or 25 ms) of 2450-MHz RFR, which increased the brain temperature by 2-4 ^{o}C.

Mitchell et al. [1977] reported an increase in motor activity on a small platform of rats exposed to 2450-MHz RFR (average SAR 2.3 W/kg, 5 hr/day, 5 days/week for 22 weeks). Motor activity of the RFR exposed rats increased during the first week of exposure and stayed higher than controls throughout the period of the experiment. Moe et al. [1976] reported a decrease in motor activity of rats exposed to RFR (918 MHz, SARs 3.6-4.2 W/kg) during the dark period of the light-dark cycle in a chronic exposure experiment (10 h/night for 3 weeks). Lovely et al. [1977] repeated the experiment using a lower intensity (2.5 mW/cm^2, SARs 0.9-1.0 W/kg, 10 h/night, 13 weeks) and found no significant change in motor activity in the exposed rats. Frey [1977] subjected rats to 1300-MHz pulsed RFR (0.5 ms pulses, 1000 pps, average power density of 0.65 or 0.2 mW/cm^2, peak power densities 1.3 and 0.4 mW/cm^2). He reported a decrease in tail pinch-induced aggressive behavior in RFR-exposed rats. Increased latency, decrease in duration, and episodes of fighting after tail pinching were observed between two rats being irradiated with RFR. Decrease in motor coordination on a motor-rod was also reported in pulsed RFR-exposed (1300 and 1500 MHz, 0.5 ms pulses, 1000 pps) rats. The effect occurred at peak power densities between 0.4 and 2.8 mW/cm^2.

Rudnev et al. [1978] studied the behavior of rats exposed to 2375-MHz RFR at 0.5 mW/cm^2 (SAR 0.1 W/kg), 7 h/day for 1 month. They reported decreases in food intake, balancing time in a treadmill and inclined rod, and motor activity in an open-field after 20 days of exposure. Interestingly, the open-field activity was found to be increased even at 3 months postexposure. In a long-term exposure study [Johnson et al., 1983], rats were exposed to pulsed 2450-MHz RFR (10 μs pulses, 800 pps) from 8 weeks to 25 months of age (22 h/day). The average whole body SAR varied as the weight of the rats increased and was between 0.4-0.15 W/kg. Open field activity was measured in 3-min sessions with an electronic open-field apparatus once every 6 weeks during the first 15 months and at 12 week intervals in the final 10 weeks of exposure. They reported a significantly lower open field activity only at the first test session and a rise in the blood corticosterone level was also observed at that time. The authors speculated that RFR might be minimally stressful to the rats.

D'Andrea et al. [1979, 1980] reported decreased motor activity on a stabilimetric platform and no significant change in running wheel activity measured overnight in rats exposed to 2450-MHz RFR (5 mW/cm^2, SAR 1.2 W/kg). However, an increase in both measurements was observed in rats exposed to 915-MHz RFR (5 mW/cm^2, SAR 2.5 W/kg). These changes in locomotor activity could be due to the thermal effect of RFR.

In a more recent experiment, Mitchell et al. [1988] studied several behavioral responses in rats after 7 h of exposure to continuous-wave 2450-MHz RFR (10 mW/cm^2, average SAR 2.7 W/kg). Decreases in motor activity and responsiveness (startle) to loud noise (8 kHz, 100 dB) were observed immediately after exposure. The rats were then trained to perform a passive avoidance task and tested for retention of the learning one week later. There was no significant difference in retention between the RFR-exposed and sham-exposed animals. The authors concluded that RFR altered responsiveness to novel environmental stimuli in the rat.

Two studies investigated the effects of pre- and postnatal-RFR on behavior. Kaplan et al. [1982] exposed groups of pregnant squirrel monkeys starting at the second trimester of pregnancy to 2450-MHz RFR at SARs of 0, 0.034, 0.34, and 3.4 W/kg (3 h/day, 5 days/week). The motor activity of the monkeys was observed at different times during the third trimester. No significant difference was observed among the different exposure groups. After birth, some dams and neonates were exposed for 6 months at the same prenatal conditions and then the offspring were exposed for another 6 months. Behavior of the mothers and offspring was observed and scored each week for the first 24 weeks postpartum. The authors observed no significant difference in maternal behavior or the general activity of the offspring among the different exposure groups. Visual-evoked EEG changes in the occipital region of the skull of the offspring were also studied at 6, 9, and 12 months of age. No significant effect of perinatal RFR-exposure was reported.

In another study [Galvin et al., 1986], rats were exposed to 2450-MHz RFR (10 mW/cm^2, 3 h/day) either prenatally (days 5-20 of gestation, whole body SAR estimated to be 2-4 W/kg) or perinatally (prenatally and on days 2-20 postnatally, whole body SARs 16.5-5.5 W/kg). Several behaviors including motor behavior, startle to acoustic and air-puff stimuli, fore- and hind-limb grip strength, negative geotaxis, reaction to thermal stimulation, and swimming endurance were studied in the rats at various times postnatally. They reported a decrease in swimming endurance (time remaining afloat in 20 ^oC water with a weight clipped to the tail) in 30-day old perinatally-exposed rats. The air-puff startle response was enhanced in magnitude in the prenatally exposed rats at 30 days, but decreased at 100 days of age. The authors concluded that perinatal exposure to RFR altered the endurance and gross motor activity in the rat. It would be interesting to study the neurochemistry or brain morphology of these animals. As described in a previous section, Albert et al. [1981a,b] and Albert and Sherif [1988] observed morphological changes in the cerebellum of rats subjected to RFR exposure perinatally at lower SAR (2-3 W/kg). It is well known that interference of cerebellar maturation can affect an animal's motor development [Altman, 1975].

O'Connor [1988] exposed pregnant rats to continuous-wave 2450-MHz (27-30 mW/cm^2) RFR between day 1 to day 18 or 19 of gestation (6 h/day). Their offspring were studied at different ages. She reported no significant effect of prenatal RFR exposure on visual cliff test, open field behavior, climbing behavior on an inclined plane, and avoidance behavior in a shuttlebox. The exposed animals showed altered sensitivity to thermally related tests evidenced by preference for the cooler section of a temperature-gradient alley way, longer latency to develop thermally induced seizure, and formed smaller huddle groups at 5 days of age.

Learned Behaviors

Many studies have investigated the effect of RFR exposure on learned behavior. King et al. [1971] used RFR as the cue in a conditioned suppression experiment. In conditioned suppression an animal is first trained to elicit a certain response (e.g., bar-press for food). Once a steady rate of response is attained, a stimulus (e.g., a tone) will signify the on-coming of a negative reinforcement (e.g., electric foot shock). The animal will soon learn the significance of the stimulus and a decrease in responding (conditioned suppression) will occur after the presentation of the stimulus. In the experiment of King et al. [1971], rats were trained to respond at a fixed-ratio schedule for sugar water reward. In a 2-h session, either a tone or RFR would be presented and occasionally followed by an electric foot shock. Radiofrequency radiation of 2450 MHz, modulated at 12 and 60 Hz and at SARs of 0.6, 1.2, 2.4, 4.8, and 6.4 W/kg were used as the conditioned stimulus. With training, consistent conditioned suppression was observed with RFR at 2.4 W/kg and higher.

Several studies used RFR as a noxious stimulus, i.e., a negative reinforcer, to induce or maintain conditioned behavior. In an earlier paper, Monahan and Ho [1976] speculated that mice exposed to RFR tended to change their body orientation in order to reduce the SAR in the body, suggesting that they were avoiding the radiation. To support the point that RFR is a noxious stimulus, Monahan and Henton [1977b] demonstrated that mice can be trained to elicit an operant response in order to escape or avoid RFR (2450-MHz, 40 W/kg).

In a series of experiments, Frey and his associates [Frey and Feld, 1975; Frey et al., 1975] demonstrated that rats spent less time in the unshielded compartment of a shuttlebox, when the box was exposed to 1200-MHz pulsed RFR (0.5 µs pulses, 1000 pps, average power density 0.2 mW/cm^2, peak power density 2.1 mW/cm^2) than during sham exposure. When a continuous-wave RFR (1200-MHz, 2.4 mW/cm^2) was used, rats showed no significant preference to remain in the shielded or unshielded side of the box. The authors also reported that rats exposed to the pulsed RFR were more active. Hjeresen et al. [1979] replicated this finding using pulsed 2880-MHz RFR (2.3 µs pulses, 100 pps, average power density 9.5 mW/cm^2) and showed that the preference to remain in the shielded side of a shuttlebox during RFR exposure could be generalized to a 37.5-kHz tone. Masking the radiation-induced auditory effect with a 10-20 kHz noise also prevented the development of shuttlebox-side preference during pulsed RFR exposure. These data suggest that the pulsed RFR-induced side preference is due to the auditory effect. In the studies of Frey et al. [1975] and Hjeresen et al. [1979] increase in motor activity was also reported when the animals were exposed to the pulsed RFR. Interestingly, this pulsed RFR-induced increase in motor activity was not affected by noise masking. Thus, the RFR avoidance and enhancement in motor activity by pulsed RFR may involve different neural mechanisms. Related to the above experiments is that the auditory effect of pulsed RFR can be used as a cue to modify an animal's behavior. Johnson et al. [1976] trained rats to respond (making nose pokes) on a fixed ratio reinforcement schedule for food pellets in the presence of a tone (7.5 kHz, 10 pps, 3 µs pulses). Reinforced period was alternated with periods of no reward when no tone was presented. Rats, after learning this response, responded when the tone was replaced by pulsed RFR (918 MHz, 10 µs pulses, 10 pps, energy per pulse 150 µJ/cm^2) during both reinforced and unrewarded periods. Apparently, the response to the tone had generalized to the pulsed RFR.

In another experiment, Carroll et al. [1980] showed that rats did not learn to go to a 'safe' area in the exposure cage in order to avoid exposure to RFR (918-MHz, pulse modulated at 60 Hz, SAR 60 W/kg), whereas the animals learned readily to escape from electric foot shock by going to the 'safe' area. In a further study, Levinson et al. [1982] showed that rats could learn to enter a 'safe' area, when the RFR (918-MHz, 60 W/kg) was paired with a light stimulus. Entering the area would turn off both the radiation and light. They also showed that rats could learn to escape by entering the 'safe' area when RFR was presented alone, but learned at a lower rate than when the RFR was paired with the light.

Several studies investigated the effect of RFR on conditioned taste aversion. It was discovered that consumption of food or drink of novel taste followed by a treatment which produced illness, e.g., X-irradiation or poison, an animal will learn to associate the taste with the illness and will later avoid the food or drink. Different from the traditional conditioning process, where conditioning occurs only when the response is followed immediately by the reinforcement, taste aversion conditioning can occur even if the illness is induced 12 h after the taste experience. Another characteristic of conditioned taste aversion is that the conditioning is very selective. An animal can learn to associate the taste with the illness, but not the place where the food or drink was taken, i.e., it will avoid the taste, but not the place where the food or drink was consumed. This phenomenon is known as 'belongingness', i.e., association (conditioning) between some stimulus pairs is easier than others [Garcia and Koelling, 1966; Garcia et al., 1966]. Thus, RFR has to produce the 'proper' type of adverse effect in the animal in order for conditioned taste aversion to occur.

Monahan and Henton [1977a] irradiated rats for 15 min with 915-MHz RFR of various intensities (up to a SAR of ~17 W/kg) after 15 min of access to 10% sucrose solution as a substitute for the normal drinking water. When the animals were offered the sucrose solution 24 h later, no conditioned taste aversion was observed. They drank the same amount of sucrose solution as the previous day. Conditioned taste aversion was also studied by Moe et al. [1976] and Lovely et al. [1977] in experiments of similar design in which rats were exposed chronically to 918-MHz RFR at 10 mW/cm^2 (SAR 3.9 W/kg) and 2.5 mW/cm^2 (SAR 1.0 W/kg), respectively. Rats were provided with 0.1% saccharin drinking solution during the whole period of exposure in the Moe et al. [1976] study and between the 9th to 13th week of exposure in the Lovely et al. [1977] study. They observed no significant difference in the consumption of saccharin solution, nor a preference for either water or saccharin solution between the RFR-exposed and sham-exposed animals. Thus, no taste aversion developed. Perhaps, RFR does not produce an intensive sickness or the proper type of 'belongingless' for the conditioning to occur. However, in another study, Lovely and Guy [1975] reported that rats that were exposed to continuous-wave 918-MHz RFR for 10 min at >25 mW/cm^2 (SAR ~22.5 W/kg) and then allowed to drink saccharin solution, showed a significant reduction in saccharin consumption when tested 24 h later. No significant effect was found in rats exposed to RFR at 5 or 20 mW/cm^2.

In addition to using RFR as an aversive stimulus, it has also been used as a positive reinforcer. Marr et al. [1988] reported that rhesus monkeys could be trained to press a lever on a fixed ratio schedule to obtain 2 sec-pulses of RFR (6500 MHz, 50 mW/cm^2, estimated SAR 12 W/kg) when the monkeys were placed in a cold environment (0 ^{o}C).

A study by Bermant et al. [1979] investigated the thermal effect of RFR using the classical conditioning paradigm. They reported that after repeated pairing of a 30 sec tone with RFR (2450 MHz, 10 sec at SAR 420 W/kg or 30 sec at SAR 220 W/kg), the tone when presented alone could elicit a conditioned hyperthermia from the rat. An effect which may be relevant to the finding of this experiment is that drug-induced changes in body temperature (hyperthermia or hypothermia) in animals can also be classically conditioned [Cunningham et al., 1984].

We have conducted experiments to investigate whether the effects of low-level RFR on psychoactive drug actions and central cholinergic activity can be classically conditioned to cues in the exposure environment. Classical conditioning of drug effects with environmental cues as the conditioned stimulus have been reported and such conditioned responses have been suggested to play a role in drug response, abuse, tolerance, and withdrawal [Le et al., 1979; Siegel, 1977, Siegel et al., 1982, Wikler, 1973a; Woods et al., 1969]. We found that the effects of RFR on amphetamine-induced hyperthermia and cholinergic activity in the brain can be classically conditioned to environmental cues [Lai et al., 1986b, 1987c].

In earlier experiments, we reported that acute (45 min) exposure to 2450-MHz RFR at average whole body SAR of 0.6 W/kg attenuated amphetamine-induced hyperthermia [Lai et al., 1983] and decreased HACU in the frontal cortex and hippocampus [Lai et al., 1987b] in the rat. In the conditioning experiments, rats were exposed to 2450-MHz pulsed RFR (2 μs pulses, 500 pps, 1.0 mW/cm^2, SAR 0.6 W/kg) in ten daily 45-min sessions. On day 11, animals were sham-exposed for 45 min and either amphetamine-induced hyperthermia or high-affinity choline uptake (HACU) in the frontal cortex and hippocampus was studied immediately after exposure. In this paradigm the RFR was the unconditioned stimulus and cues in the exposure environment were the neutral stimuli, which after repeated pairing with the unconditioned stimulus became the conditioned stimulus. Thus on the 11th day when the animals were sham-exposed, the conditioned stimulus (cues in the environment) alone would elicit a conditioned response in the animals. In the case of amphetamine-induced hyperthermia [Lai et al., 1986b], we observed a potentiation of the hyperthermia in the rats after the sham exposure. Thus, the conditioned response (potentiation) was opposite to the unconditioned response (attenuation) to RFR. This is known as 'paradoxical conditioning' and is seen in many instances of classical conditioning [cf. Mackintosh, 1974]. In addition, we found in the same experiment that, similar to the unconditioned response, the conditioned response could be blocked by the drug naloxone, implying the involvement of endogenous opioids. In the case of RFR-induced changes in cholinergic activity in the brain, we [Lai et al., 1987c] found that conditioned effects also occurred in the brain of the rat after the session of sham exposure on day 11. An increase in HACU in the hippocampus (paradoxical conditioning) and a decrease in the frontal cortex were observed. In addition, we found that the effect of RFR on hippocampal HACU habituated after 10 sessions of exposure, i.e., no significant change in HACU in the hippocampus was observed in animals exposed to the RFR on day 11. On the other hand, the effect of RFR on frontal cortical HACU did not habituate after the repeated exposure.

An explanation for the paradoxical conditioning phenomenon was given by Wikler [1973b] and Eikelboom and Stewart [1982]. The direction of the conditioned response (same as or opposite to the unconditioned response) depends on the site of action of the unconditioned stimulus, whether it is on the afferent or efferent side of the affected neural feedback system. Thus, in order to further understand the neural mechanisms of the conditioned effects, the site of action of RFR on the central nervous system has to be identified.

Little work has been done to investigate the effects of RFR on memory functions. We [Lai et al., 1989b] studied the effect of acute (20 or 45 min) RFR exposure (2450-MHz, 1 mW/cm^2, SAR 0.6W/kg) on the rats' performance in a radial-arm maze, which measures spatial learning and memory functions. The maze consists of a central circular hub with arms radiating out like the spokes of a wheel. In this task, food-deprived animals are trained to explore the arms of the maze to obtain food reinforcement at the end of each arm. In each session they have to enter each arm once and a reentry is considered as an error. This task requires the so called 'working memory', i.e., the rat has to remember the arms it has already entered during the course of a session. Working memory requires the functions of the cholinergic innervations in the frontal cortex and hippocampus [Dekker et al., 1991; Levin, 1988]. Both have been shown to be affected by acute RFR exposure [Lai et al., 1987b]. We [Lai et al., 1989b] found that acute (45 min) exposure to RFR before each session of maze running significantly retarded the rats' abilities to perform in the maze. They made significantly more errors than the sham-exposed rats. This result agrees with the neurochemical finding that 45 min of RFR exposure decreased the activity of the cholinergic systems in the frontal cortex and hippocampus of the rats [Lai et al., 1987b]. However, 20 min of RFR exposure, which increased cholinergic activity in the brain, did not significantly affect maze performance. Apparently, increase in cholinergic activity cannot further improve the performance, since the neural systems involved in the memory function may be working at optimal levels under normal conditions. In a recent experiment [Lai et al., 1993], we have shown that the microwave-induced working memory deficit in the radial-arm maze was reversed by pretreating the rats before exposure with the cholinergic agonist physostigmine or the opiate antagonist naltrexone, whereas pretreatment with the peripheral opiate antagonist naloxone methiodide showed no reversal of effect. These data indicate that both cholinergic and endogenous opioid neurotransmitter sysatems inside the central nervous system are involved in the microwave-induced spatial memory deficit.

Several studies have investigated the effect of RFR on discrimination learning and responding. Hunt et al. [1975] trained rats to bar press for saccharin water rewards in the presence (5 sec duration) of a flashing light and not to respond in the presence of a tone (unrewarded). After 30 min of exposure to 2450-MHz RFR, modulated at 20 Hz and at SAR of 6.5 or 11.0 W/kg, rats made more misses at the presence of the light, but there were no significant changes in the incidences of bar-pressing errors when the tone was on. The effect was more prominent at the higher dose rate. Galloway [1975] trained rhesus monkeys on two behavioral tasks to obtain food reward. One was a discrimination task in which the monkey had to respond appropriately depending on which of the two stimuli was presented. The other task was a repeated acquisition task in which a new sequence of responses had to be learned everyday. After training, the animals were irradiated with continuous-wave 2450-MHz RFR applied to the head prior to each subsequent behavioral session. The integral dose rates varied from 5-25 W. Some of these dose rates caused convulsions in the monkeys. The radiation was shown to exert no significant effect on the discrimination task, whereas a dose-dependent deficit in performance was observed in the repeated acquisition task. Cunitz et al., [1979] trained two rhesus monkeys to move a lever in different directions depending on the lighting conditions in the exposure cage in order to obtain food reinforcement on a fixed ratio schedule. After the animals' performance had reached a steady and consistent level, they were irradiated at the head with continuous-wave 383-MHz RFR at different intensities in subsequent sessions. Radiation started 60 min before and during a session of responding. The authors reported a decrease in the rate of correct responding when the SAR at the head reached 22-23 W/kg. In another study, Scholl and Allen [1979] exposed rhesus monkeys to continuous-wave 1200-MHz RFR at SARs of 0.8-1.6 W/kg and observed no significant effect of the radiation on a visual tracking task.

de Lorge [1976] trained rhesus moneys on an auditory vigilance (observing-response) task. The task required continuous sensory-motor activities in which the monkeys had to coordinate their motor responses according to the stimulus cues presented. In the task the monkeys had to press the right lever that produced either a 1070-Hz tone for 0.5 sec or a 2740-Hz tone. The 1070-Hz tone signalled an unrewarded situation. Pressing a left lever when the 2740-Hz tone was on would produce a food reward. Presentation of the higher frequency tone was on a variable interval schedule. After the monkeys had learned to perform the task at a steady level, they were irradiated with 2450-MHz RFR of different intensities. Decreased performance and increased latency time in pressing the left lever were observed when the power density at the head was at 72 mW/cm^2. The deficits could be due to an increase in colonic temperature after exposure to the high intensity RFR.

de Lorge [1979] trained squirrel monkeys to respond to another observing-response task using visual cues. After learning the task, the animals were exposed to 2450-MHz RFR (sinusoidally modulated at 120 Hz) for 30 or 60 min at different power densities (10-75 mW/cm^2) in subsequent sessions. Their performances were disrupted at power densities >50 mW/cm^2. The disruption was power density-dependent and occurred when the rectal temperatures increased more than 1 ^{o}C. In a more recent experiment, de Lorge [1984] studied rhesus monkeys trained on the auditory vigilance task and the effects of exposure to RFRs of different frequencies (225, 1300, and 5800 MHz). Reduction in performance was observed at different power density thresholds for the frequencies studied: 8.1 mW/cm^2 (SAR 3.2 W/kg) for 225 MHz, 57 mW/cm^2 (SAR 7.4 W/kg) for 1300 MHz, and 140 mW/cm^2 (SAR 4.3 W/kg) for 5800 MHz. de Lorge concluded that the behavioral disruption under different frequencies of exposure was more correlated with change in body temperature. Disruption occurred when the colonic temperature of the animal had increased by 1 ^{o}C.

Many studies have investigated the effects of RFR on reinforcement schedule-controlled behavior. Sanza and de Lorge [1977] trained rats on a fixed interval schedule for food pellets. After 60 min of exposure to 2450-MHz RFR (modulated at 120 Hz) at 37.5 mW/cm^2, a decrease in response with an abrupt onset was observed. This effect was more pronounced in rats with a high base line of response rate on the fixed interval schedule. No significant effect on response was observed at power densities of 8.8 and 18.4 mW/cm^2.

D'Andrea et al. [1976] trained rats to bar-press for food at a variable interval schedule. After a constant responding rate was attained, the animals were irradiated with continuous- wave RFRs of 360, 480, or 500 MHz. Bar-press rates were decreased only when the rats were exposed to the 500-MHz radiation at a SAR of approximately 10 W/kg. The animals also showed significant signs of heat stress. In a subsequent study [D'Andrea et al., 1977] RFRs of different frequencies and intensities were studied on their effect on bar-pressing rate on a variable interval schedule. It was found that the latency time of stoppage to respond after the radiation was turned on correlated with the rate of rise in body temperature of the animal. These experiments definitely demonstrated the thermal effect of RFR on operant behavior.

Gage [1979a] trained rats on a variable interval schedule for food reinforcement. Different groups of rats were exposed overnight (15 h) to continuous-wave 2450-MHz RFR at either 5, 10, or 15 mW/cm^2. Responses were tested immediately after exposure. No significant difference in performance was found between the RFR- and sham-exposed rats when exposure was done at an ambient temperature of 22 ^{o}C. However, a power density- dependent reduction in response rate and increase in response duration was found in the RFR-exposed rats when the irradiation was carried out at 28 ^{o}C. At the higher ambient temperature, heat dissipation from the body was less efficient and the exposed rats had higher body temperatures postexposure.

Lebovitz [1980] also studied the effects of pulsed 1300-MHz (1 μs pulses, 600 pps) RFR on rats bar-pressing on a fixed interval schedule for food reinforcement. Both food reinforced bar presses and unrewarded bar presses during the intervals were studied. No significant effect was detected in both types of response at SAR of 1.5 W/kg. However, at 6 W/kg, there was a slight reduction in rewarded bar presses and a large reduction in unrewarded bar presses. The authors concluded that the unrewarded behavior was more susceptible to the effect of RFR than the rewarded behavior. Another related experiment was reported by Sagan and Medici [1979] in which water-deprived chicks were given access to water on fixed intervals irrespective of their responses. During the time between water presentations the chicks showed an increase in motor activity known as 'interim behavior'. Exposure to 450-MHz RFR amplitude-modulated at 3 and 16 Hz at power densities of either 1 or 5 mW/cm^2 during session had no significant effect on the 'interim behavior'.

Effects of RFR on complex operant response sequence and reinforcement schedules were studied in various experiments. de Lorge and Ezell [1980] tested rats on a vigilance behavioral task during exposure to pulsed 5620-MHz RFR and then to pulsed 1280-MHz RFR. In this task, rats had to discriminate two tones in order to press one of two bars appropriately for food reinforcement. Behavioral decrement was observed at an SAR of 2.5 W/kg with the 1280-MHz radiation, but at 4.9 W/kg with the 5620-MHz radiation. Gage [1979b] trained rats to alternate responses between 2 levers at 11-30 times for a food reinforcement. Decrement in response rates was observed after 15 h of exposure to continuous-wave 2450-MHz RFR at 10, 15, and 20 mW/cm^2 (0.3 W/kg per mW/cm^2).

Thomas et al. [1975] trained rats to bar press on two bars: a fixed ratio of 20 on the right bar (20 bar presses produced a food pellet reward) and differential reinforcement of low rate (DRL) on the left bar (bar presses had to be separated by at least 18 sec and no more than 24 sec to produce a reward). There was a time-out period between schedules, i.e., no reinforcement available for responding. Animals were tested 5-10 min after 30 min of exposure to either continuous-wave 2450-MHz, pulsed 2860-MHz (1 μs pulses, 500 pps) or pulsed 9600-MHz (1 μs pulses, 500 pps) RFR at various power densities. An increase in DRL response rate was observed with 2450-MHz radiation >7.5 mW/cm^2 (SAR 2.0 W/kg), 2860-MHz RFR >10 mW/cm^2 (2.7 W/kg), and 9600-MHz RFR >5 mW/cm^2 (SAR 1.5 W/kg). A decrease in the rate of response at the fixed ratio schedule was seen in all three frequencies when the power density was greater than 5 mW/cm^2. In addition, an increase in response rate was observed during time-out periods under irradiation of the three frequencies of RFR at greater than 5 mW/cm^2.

In another study, Thomas et al. [1976] trained rats to bar press on a tandem schedule using 2 bars. Pressing the right bar for at least 8 times before pressing the left bar would give a food pellet reward. A power density-dependent decrease in the percentage of making 8 or more consecutive responses on the right bar before pressing the left bar was observed in the animals after 30 min of exposure to pulsed 2450-MHz RFR (1 μs pulses, 500 pps) at power densities of 5, 10, and 15 mW/cm^2.

Schrot et al [1980] also trained rats to learn a new daily sequence of pressing of three bars for food reinforcement. An increased number of errors and decreased learning rates were observed in the animals after 30 min of exposure to pulsed 2800-MHz RFR (2 μs pulses, 500 pps) at average power densities of 5 and 10 mW/cm^2 (SARs 0.7 and 1.7 W/kg, respectively). No significant effect on performance was observed at power densities of 0.25, 0.5, and 1 mW/cm^2.

Several studies investigated the effects of chronic RFR exposure on schedule controlled-behavior. Mitchell et al. [1977] trained rats to respond on a mixed schedule of reinforcement

(FR-5 EXT-15 sec), in which 5 responses would give a reward and then a 15 sec lapse time (extinction period) was required before a new response would be rewarded. In addition, the schedule of reinforcement was effective when a lamp was on, while no reinforcement was given when the lamp was off. Rats were then exposed to 2450-MHz RFR (average SAR 2.3 W/kg) for 22 weeks (5 h/day, 5 days/week) and tested at different times during the exposure period. The RFR-exposed rats showed higher responses during the extinction period, indicating poorer discrimination of the response cues. In another also pretrained task, rats had to press a bar to postpone the onset of unsignalled electric foot-shocks (unsignalled avoidance paradigm). No significant difference in performance of this task was observed between the RFR- and sham-exposed animals.

Two series of well-designed experiments were run by D'Andrea et al. [1986a,b] to investigate the effects of chronic RFR exposure on behavior. In one experiment, rats were exposed for 14 weeks (7 h/day, 7 days/week) to continuous-wave 2450-MHz RFR at 2.5 mW/cm^2 (SAR 0.7 W/kg). Decrease in the threshold of electric foot shock detection (i.e., increase in sensitivity) was observed in the irradiated rats during the exposure period. Increased open-field exploratory behavior was observed in the rats at 30 days postexposure. After exposure, the rats were trained to bar press on an interresponse time criterion (IRT). In this schedule, the animals had to respond within 12 to 18 sec after the previous response in order to receive a food reward. Radiofrequency radiation exposed rats emitted more responses during the training period. When the training was completed, the RFR-exposed rats had lower efficiency in bar-pressing to obtain food pellets, i.e., they made more inappropriate responses and received fewer food pellets than the sham-exposed rats during a session. In a signalled two-way active avoidance shuttlebox test, the RFR-exposed rats showed less avoidance response than the sham-exposed rats during training; however, no significant difference in responses in the shuttlebox test was detected at 60 days after exposure between the RFR- and sham-exposed animals. In another series of experiments, rats were exposed to 2450-MHz RFR at 0.5 mW/cm^2 (SAR 0.14 W/kg) for 90 days (7 h/day, 7 days/week). Open-field behavior, shuttlebox performance, and IRT schedule-controlled bar-pressing behavior for food pellets were studied at the end of the exposure period. A small deficit in shuttlebox performance and increased rate of bar-pressing were observed in the RFR exposed rats. Summarizing the data from these two series of experiments [D'Andrea et al., 1986a,b], D'Andrea and his co-workers concluded that the threshold for the behavioral and physiological effects of chronic RFR exposure in the rats studied in their experiments occurred between the power densities of 0.5 mW/cm^2 (SAR 0.14 W/kg) and 2.5 mW/cm^2 (SAR 0.7 W/kg).

D'Andrea et al. [1989] recently studied the behavioral effects of high peak power RFR pulses of 1360-MHz. Rhesus monkeys performing on a complicated reinforcement-schedule involving time-related behavioral tasks (inter-response time, time discrimination, and fixed interval responses) were exposed to high peak power RFR (131.8 W/cm^2 rms, pulse repetition rate 2-32 Hz). No significant disturbance in performance was observed in the monkeys.

Akyel et al. [1991] also studied the effects of exposure to high peak power RFR pulses on behavior. In their experiment, rats pretrained to bar-press for food reinforcement on either fixed ratio, variable interval, or DRL schedule were exposed for 10 min to 1250-MHz pulses. Each pulse (10 μs width) generated a whole body specific absorption of 2.1 J/kg, which corresponds to a whole body average SAR of 0.21 mW/kg. The pulse rate was adjusted to produce different total doses (0.5-14 kJ/kg). Only at the highest dose (14 kJ/kg), stoppage of responding was observed after exposure, when the colonic temperature was increased by ~2.5 ^{o}C. Responding resumed when colonic temperature returned to within 1.1 °C above the preexposure level. When responding resumed, the response rates on the fixed ratio and variable interval schedules were below the preexposure base line level. Responses on the DRL schedule were too variable to allow a conclusion to be drawn. The authors concluded that the effect of the high peak power RFR pulses on schedule-controlled behavior was due to hyperthermia.

Behavior conditioning using different reinforcement schedules generates stable base line responses with reproducible patterns and rates. The behavior can be maintained over a long period of time (hrs) and across different experimental sessions. Thus, schedule-controlled behavior provides a powerful means for the study of RFR-behavior interaction in animals. On the other hand, the behavior involves complex stimulus-response interactions. It is difficult to conclude from the effects of RFR on schedule-controlled behavior the underlying neural mechanisms involved.

In a sense, these studies of RFR are similar to those of psychoactive drugs. A large volume of literature is available on the latter topic. A review of the literature on the effects of psychoactive drugs on schedule-controlled behavior reveals the complexity of the interaction and the limitation in data interpretation. In general, the effects of psychoactive drugs on schedule-controlled behavior is dose-dependent. In many cases, especially in behavior maintained by positive reinforcement, an inverted-U-function has been reported, i.e., the behavior is increased at low doses and decreased at high doses of the drug. In addition, the way that a certain drug affects schedule-controlled behavior depends on three main factors: (a) the base line level and pattern of responding of the animal: a general rule is that drugs tend to decrease the rate when the base line responding rate is high and vice versa. This is known as rate-dependency and is true with psychomotor stimulants, major and minor tranquilizers, sedative-hypnotics, and narcotics; (b) the schedule of reinforcement: in addition to its effect on the base line responding rate, a reinforcement schedule can have other specific effects on responses. For example, amphetamine has different effects on responses maintained on DRL schedule and punishment-suppressed responding schedule, even though both schedules generate a similar low response rate; and (c) the stimulus-control involved in the study: e.g., responses maintained by electric shock are more resistant to drug effects than responses maintained by positive reinforcers. On the other hand, some drugs have differential effects on signalled-avoidance versus continuous avoidance responding.

Thus, to fully understand the effect of RFR, the parameters of the radiation (different dose rates, frequency, duration of exposure, etc.), different reinforcement-schedules, and conditioning procedures have to be carefully studied and considered. However, there is evidence that the above determining factors on schedule-controlled behavior may also hold in the case of RFR. Exposure to RFR caused a decrease in response rate when a variable interval schedule that produces a steady rate of responding was used [D'Andrea et al., 1976; 1977; Gage, 1979a], and an increase in responding when the DRL-schedule of reinforcement was used [Thomas et al., 1975]. This may reflect the rate-dependency effect. On the other hand, stimulus control as a determinant of response outcome was seen in the study of Lebovitz [1980] when unrewarded responses were disrupted more by RFR than rewarded responses, and the study of Hunt et al. [1975] that showed the reverse relationship. In the former experiment a fixed interval schedule was used, whereas in the latter a discrimination paradigm was studied.

Another related point is that most psychoactive drugs affect body temperature. Stimulants cause hyperthermia, barbiturates cause hypothermia, and narcotics have a biphasic effect on body temperature (hyperthermia at low doses and hypothermia at high doses). It is not uncommon to observe a change of 2-3 °C within 30 min after a drug is administered. However, in reviewing the literature, there is no general correlation between the effects of the drugs on body temperature and schedule-controlled behavior. Thus, body temperature may not be an important factor in an animal's responding under schedule-controlled behavior, at least in the case of psychoactive drugs. On the contrary, some of the experiments described above strongly suggest the role of hyperthermia on the RFR effect on the behavior. Perhaps, a sudden and large increase in body temperature as in the case of RFR can have a major effect on responding.

Generally speaking, when effects were observed, RFR disrupted operant behavior in animals such as in the cases of discrimination responding [de Lorge and Ezell, 1980; Hunt et al., 1975; Mitchell et al., 1977], learning [Lai, 1989b; Schrot et al., 1980], and avoidance [D'Andrea et al., 1986a,b]. This is especially true when the task involved complex schedules and response sequence. In no case has an improvement in operant behavior been reported after RFR exposure. It is interesting that only disruptions in behavior by RFR exposure are reported. In the studies on EEG, both excitation (desynchronization) and depression (synchronization) have been reported after exposure to RFR [Bawin et al., 1979; Chizhenkova, 1988; Chou et al., 1982b; Dumansky and Shandala, 1976; Goldstein and Sisko, 1974; Dumansky and Shandala, 1976; Takeshima et al., 1979]. Motor activity has also been reported to increase [D'Andrea et al., 1979, 1980; Hunt et al., 1975; Mitchell et al., 1977; Rudnev et al., 1978] and decrease [Johnson et al., 1983; Mitchell et al., 1988; Moe et al., 1976; Rudnev et al., 1978] after RFR exposure. If these measurements can be considered as indications of electrophysiological and behavioral arousal and depression, improvement in operant behavior should occur under certain conditions of RFR exposure. This is especially true with avoidance behavior. Psychomotor stimulants that cause EEG desynchronization and motor activation improve avoidance behavior, whereas tranquilizers that have opposite effects on EEG and motor activity decrease avoidance behavior.

GENERAL DISCUSSION

After reviewing the studies on the effects of RFR on the central nervous system, one obvious question comes to my mind: "What is the mechanism responsible for the effects reported?" In most cases, especially the in vivo studies in which high intensities of irradiation were used resulting in an increase in body temperature, thermal effect is most likely the answer. Even in cases when no significant change in body temperature was detected, thermal effect cannot be excluded. An animal can maintain its body temperature by actively dissipating the heat load from the radiation. Activation of thermoregulatory mechanisms can lead to neurochemical, physiological, and behavioral changes. Temperature can be better controlled during in vitro studies. Uneven heating of the sample can still generate temperature gradients, which may affect the normal responses of the specimen studied. However, several points raised by some experiments suggest that the answer is not a simple one. They are: (a) 'Heating controls' do not produce the same effect of RFR [D'Inzeo et al., 1988; Seaman and Wachtel, 1978; Synder, 1971; Johnson and Guy, 1971; Wachtel et al., 1975]; (b) Window effects are reported [Bawin et al., 1975, 1979; Blackman et al., 1979, 1980a,b, 1989; Chang et al., 1982; Dutta et al., 1984, 1989, 1992; Lin-Liu and Adey, l982; Oscar and Hawkins, 1977; Sheppard et al., 1979]; (c) Modulated or pulsed RFR is more effective in causing an effect or elicits a different effect when compared with continuous-wave radiation of the same frequency [Arber and Lin, 1985; Baranski, 1972; Frey et al., 1973, 1975; Oscar and Hawkins, 1977; Sanders et al., 1983]; (d) Different frequencies of RFR produce different effects [D'Andrea et al., 1979, 1985; de Lorge and Ezell, 1980; Sanders et al., 1984; Thomas et al., 1975]; and (e) Different exposure orientations or systems of exposure produce different effects at the same average whole body SAR [Lai et al., 1984a, 1988].

I think most of these effects can be explained by the following factors:

1. The physical properties of RFR absorption in the body and the mechanisms by which RFR affects biological functions were not fully understood. In addition, use of different exposure conditions make it difficult to compare the results from different experiments.
2. Characteristics of the response system, i.e., the dependent variable, were not fully understood. In many cases, the underlying mechanism of the response system studied was not known.
3. Dose-response relationship was not established in many instances and conclusions were drawn from a single RFR intensity or exposure duration.

It is well known that the distribution of RFR in an exposed object depends on many factors such as frequency, orientation of exposure, dielectric constant of the tissue, etc. D'Andrea et al. [1987] and McRee and Davis [1984] pointed out the uneven distribution of energy absorbed in the body of an exposed animal with the existence of 'hot spots'. In experiments studying the central nervous system, Williams et al. [1984d] also reported a temperature gradient in the brain of rats exposed to RFR. Structures located in the center of the brain, such as the hypothalamus and medulla, had higher temperatures than peripheral locations, such as the cerebral cortex. In a study by Chou et al. [1985a], comparisons were made of the local SARs in eight brain sites of rats exposed under seven exposure conditions, including exposure in a circular waveguide with the head or tail of an animal facing the radiation source, near field and far field exposures with either E- or H-field parallel to the long-axis of the body, and dorsal exposure in a miniature anechoic chamber with E- or H-field parallel to the long axis of the body. Statistical analysis of the data showed that a) there was a significant difference in local SARs in the eight brain regions measured under each exposure condition, and b) the pattern of energy absorption in different regions of the brain depended on the exposure condition. However, it must be pointed out that in another study [Ward et al., 1986], no temperature 'hot spots' were detected in the brains of rat carcasses and anesthetized rats after irradiation with 2450-MHz RFR. Temperature increases in various regions of the brain were found to be uniform and dependent on the power density of the radiation.

A question that one might ask is whether different absorption patterns in the brain or body could elicit different biological responses in the animal. If this is positive, possible outcomes from the study of bioelectromagnetics research are: (1) a response will be elicited by some exposure conditions and not by others, and (2) different response patterns are elicited by different exposure conditions, even though the average dose rates in the conditions are equal. We [Lai et al., 1984a] reported a difference in responses to the hypothermic effects of pentobarbital depending on whether the rat was exposed with its head facing toward or away from the source of radiation in the waveguide with the average whole body SAR under both conditions remaining the same; however, the patterns of energy absorption in the body and the brain differed in the two exposure conditions. Studies of HACU activity in the different regions of the brain [Lai et al., 1988] also showed that different responses could be triggered using different exposure systems or different waveforms of RFR (continuous-wave or pulsed) with the average whole body SAR held constant under each exposure condition. These data indicate that the energy distribution in the body and other properties of the radiation can be important factors in determining the outcome of the biological effects of RFR. A series of studies by Frei et al. [1989a,b] also demonstrated some interesting results on this issue. The effects of high intensity 2450- and 2800-MHz RFRs on heart rate, blood pressure, and respiratory rate in ketamine-anesthetized rats were studied. Both frequencies produced increases in heart rate and blood pressure and no significant difference was observed whether continuous-wave or pulsed radiation was used. A difference was observed, however, when the animals were exposed with their bodies parallel to the H- or E-field. In the case of 2450-MHz RFR, the E-orientation exposure produced greater increases in heart rate and blood pressure than the H-orientation exposure; whereas no significant difference in the effects between the two exposure orientations was observed with the 2800-MHz radiation. The authors speculated that the differences could be attributed to the higher subcutaneous temperature and faster rise in colonic temperature in the E-orientation when the rats were exposed at 2450 MHz than at 2800 MHz. Once again, this points out that subtle differences in exposure parameters could lead to different responses. Therefore, due to the peculiar pattern of energy deposition and heating by RFR, it may be impossible to replicate the thermal effect of RFR by general heating, i.e., use of temperature controls.

The fact that dosimetry data were based on stationary models that usually show discrete patterns of energy absorption, further complicate the matter. In animal studies, unless the animal is restrained, the energy absorption pattern changes during the exposure period depending on the position and the orientation of the animal. A possible solution would be to perform long-term exposure experiments, thus, the absorption pattern on the average would be made more uniform.

Another important consideration regarding the biological effects of RFR is the duration or number of exposure episodes. This is demonstrated by the results of some of the studies on the neurological effects of RFR. Depending on the responses studied in the experiments, several outcomes could result: an effect was observed only after prolonged (or repeated) exposure, but not after acute exposure [Baranski, 1972; Baranski and Edelwejn, 1968, 1974; Mitchell et al., 1977; Takashima et al., 1979], an effect disappeared after prolonged exposure suggesting habituation [Johnson et al., 1983; Lai et al., 1987c, 1992a], and different effects were observed after different durations of exposure [Baranski, 1972; Dumanski and Shandala, 1974; Grin, 1974; Lai et al., 1989a, 1989b; Servantie et al., 1974; Snyder, 1971]. All of these different responses reported can be explained as being due to the different characteristics of the dependent variable studied. An interesting question related to this is whether or not intensity and duration of exposure interact, e.g., can exposure to a low intensity over a long duration produce the same effect as exposure to a high intensity radiation for a shorter period?

Thus, even though the pattern or duration of RFR exposure is well-defined, the response of the biological system studied will still be unpredictable if we lack sufficient knowledge of the response system. In most experiments on the neurological effects of RFR, the underlying mechanism of the dependent variable was not fully understood. The purpose of most of the studies was to identify and characterize possible effects of RFR rather than the underlying mechanisms responsible for the effects. This lack of knowledge of the response system studied is not uncommon in biological research. In this regard, it may be appropriate to compare the biological and neurological effects of RFR with those of ethanol. Both entities exert non-specific effects on multiple organs in the body. Their effects are nonspecific, because both ethanol and RFR are not acting on specific receptors. The biological effects of ethanol could be a general action on cell membrane fluidity.

In reviewing the literature on the neurological effects of ethanol, one notices some similarity with those of RFR. In both cases, a wide variety of neurological processes were reported to be affected after exposure, but without a known mechanism. On the other hand, inconsistent data were commonly found. For example, in the case of the effects of ethanol on dopamine receptors in the brain, an increase [Hruska, 1988; Lai et al., 1980], a decrease [Lucchi et al., 1988; Syvalahti et al., 1988], and no significant change [Muller, 1980; Tabakoff and Hoffman, 1979] in receptor concentration have been reported by different investigators. Such inconsistencies have existed since the late 70's and there has been no satisfactory explanation for them. Similar research findings of increase, decrease, and no significant change in the concentration of muscarinic cholinergic receptors in the cerebral cortex of animals treated with ethanol have also been reported in the literature [Kuriyama and Ohkuma, 1990]. Dosage and route of ethanol treatment, the frequency of administration, and the species of animal studied, etc., could all attribute to variations in the findings [Keane and Leonard, 1989]. As we have discussed earlier, such considerations on the parameters of treatment also apply to the study of the biological effects of RFR. These are further complicated by the special properties of the radiation, such as waveform and modulation. In addition, RFR effects could have rapid onset and offset when the source was turned on and off, whereas the biological effect of ethanol depends on the rates of absorption and metabolism.

Thus, an understanding of the response characteristics of the dependent variables to different parameters of RFR, such as power density, frequency, waveform, etc., is important. Lack of knowledge about such characteristics may explain some of the discrepancies in bioelectromagnetics research results in the literature. Non-linear response characteristics are frequently observed in biological systems, because different mechanisms are involved in producing a response. For example, in the case of apomorphine-induced locomotor activity, a low dose of apomorphine (e.g., 0.1 mg/kg) decreases locomotor activity, whereas a higher dosage (e.g., 1.0 mg/kg) of the drug causes a profound enhancement. A dose in between may cause an insignificant effect. An explanation for this phenomenon is that a low dose of apomorphine activates selectively presynaptic dopamine receptors in the brain, which decreases dopamine release from its terminals and, thus, a decrease in motor activity. At a high dose, apomorphine stimulates the postsynaptic dopamine receptors, leading to an increase in motor activity.

Another common response-characteristic is the inverted-U function. In this situation, a response is only seen at a certain dose range and not at higher or lower dosages. An example of an inverted-U dose-response function is the effect of benzodiazepines on schedule controlled operant behavior. There is not a good explanation for the occurrence of this function. One possible explanation might be that at least two mechanisms, a facilitatory and an inhibitory function, are involved in the response. At a lower dose range of the drug, for example, the facilitatory mechanism predominates and leads to enhancement of the response, whereas, as the dosage increases an inhibitory mechanism is activated, leading to a decline in response. Thus, it is essential that the dose-response function be determined.

The inverted-U response-characteristic can be the basis of some of the 'window' effects reported in bioelectromagnetics research. Thus, with the above considerations, it is not surprising that RFR can cause enhancement, decrement, and no significant effect on a particular response depending upon the exposure conditions. Blackman et al. [1991] stated on the effect of temperature on calcium ion efflux from brain tissue that, "... either outcome (*inhibition or enhancement in release of calcium ions*), or a null result, is possible, depending on the temperature of tissue sample before and during exposure". However, it must be pointed out that the inverted-U function is not sufficient to account for the 'multiple window' effect reported in one of Blackman's studies [Blackman et al., 1989].

Another important consideration in the study of the central nervous system should be mentioned here. It is well known that the functions of the central nervous system can be affected by activity in the peripheral nervous system. Thirty years ago, McAfee [1961, 1963] pointed out that the thermal effect of RFR on the peripheral nervous system can lead to changes in central nervous system functions and behavior in the exposed animal. This is especially important in the in vivo experiments when the whole body is exposed. However, in most experiments studying the effects of RFR on the central nervous system, the possibility of contribution from the peripheral nervous system was not excluded in the experimental design. Therefore, caution should be taken in concluding that a neurological effect resulted solely from the action of RFR on the central nervous system.

An interesting question arose, whether or not RFR could produce 'non-thermal' biological effects. Many have speculated whether RFR can directly affect the activity of excitable tissues. Schwan [1971, 1977] pointed out that it would take a very high intensity of RFR to directly affect the electrical activity of a cell. On the other hand, Wachtel et al. [1975] have speculated that an RFR-induced polarized current in the membrane of a neuron could lead to changes in activity. Adey [1988] has suggested that cooperative processes in the cell membrane might be reactive to the low energy of oscillating electromagnetic field, leading to a change in membrane potential. Pickard and Barsoum [1988] recorded from cells of the Characeae plant exposed to 0.1-5 MHz pulsed RFR and observed a slow and fast component of change in membrane potential. The slow component was temperature dependent and the fast component was suggested to be produced by rectification of the oscillating electric field induced by RFR on the cell membrane. However, the effect disappeared when the frequency of radiation reached ~10 MHz.

An extreme example of the direct interaction of electromagnetic radiation with a specific biological molecule triggering a neurological effect is the rhodopsin molecules in the rod photorecepter cells that transduce light energy into neural signals. In 1943, a psychophysical experiment by Hecht et al. [1942] suggested that a single photon could activate a rod cell. The molecular biology of rhodopsin is now well understood. It is now known that a single photon can activate a single molecule of rhodopsin. A photon of the visible spectrum turns 11-cis retinol, a moiety of the rhodopsin molecule, to an all-trans form. This triggers a cascade of molecular activities involving specific G-protein, the conversion of cyclic-GMP to 5'-GMP, and eventually closing the sodium-ion channels on the cell membrane of the rod cell. This cascade action leads to a powerful amplification of the photon signal. It was estimated that one photon can affect several hundred C-GMP molecules. Such change is enough to hyperpolarize a rod cell and lead to signal transmission through its synapse [Liebman et al., 1987; Stryer, 1987]. Can a similar molecular sensitive to RFR exist? The problem is that RFR energy is several orders of magnitude (~10^6) lower than that of a photon at the visual spectrum. It is difficult to visualize a similar molecular mechanism sensitive enough to detect RFR.

Another consideration is that the ambient level of RFR is very low in the natural environment and could not have generated enough selection pressure for the evolutionary development of such a molecular mechanism. On the other hand, there may be some reason for the development of a molecular mechanism for the detection of static or low frequency electric or magnetic fields. An example is the electroreception mechanism of two Australian monotremes, the platypus, *Ornithorhynchus anatinus*, and the echidna, *Tachyglossus aculeatus* [Gregory et al., 1989a,b; Iggo et al., 1992; Scheich et al., 1986]. Apparently, receptors sensitive to low-level electric fields exist in the snout and bill of these animals, respectively. Electrophysiological recordings from the platypus show that receptors in the bill can be sensitive to a static or sinusoidally changing (12-300 Hz) electric field of 4-20 mV/cm, and cells in the cerebral cortex can respond to a threshold field of 300 μV/cm. Moreover, behavioral experiments showed that the platypus can detect electric fields as small as 50 μV/cm. In the echidna snout, receptors can respond to fields of 1.8-73 mV/cm. These neural mechanisms enable the animals to detect muscular movements of their prey, termites and shrimps. It would be interesting to understand the transduction mechanism in the electroreceptors in these animals. However, it remains to be seen whether RFR can generate a static or ELF field in tissue and that a similar electroreceptor mechanism exists in other mammals.

Another possible explanation suggested for the neurological effects of RFR is stress. This hypothesis has been proposed by Justesen et al. [1973] and Lu et al. [1980] and based on high intensity of exposure. We have also proposed recently that low-level RFR may be a 'stressor' [Lai et al., 1987a]. Our speculation is based on the similarity of the neurological effects of known stressors (e.g., body-restraint, extreme ambient temperature) and those of RFR (see Table 1 in Lai et al., 1987a). Our recent experiments suggesting that low-level RFR activates both endogenous opioids and corticotropin-releasing factor in the brain further support this hypothesis. Both neurochemicals are known to play important roles in an animal's responses to stressors [Amir et al., 1980; Fisher, 1989]. However, it is difficult to prove that an entity is a stressor, since the criteria of stress are not well defined and the caveat of stress is so generalized that it has little predictive power on an animal's response.

In conclusion, I believe the questions on the biological effects of RFR and the discrepancies in research results in the literature can be resolved by (a) a careful and thorough examination of the effects of the different radiation parameters, and (b) a better understanding of the underlying mechanisms involved in the responses studied. With these considerations, it is very unlikely that the neurological effects of RFR can be accounted for by a single unifying neural mechanism.

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Appendix 9-B - Memory and Behavior

Presention: The Biological Effects, Health Consequences and Standards for Pulsed Radiofrequency Field. International Commission on Nonionizing Radiation Protection and the World Health Organization, Ettoll Majorare, Centre for Scientific Culture, Italy, 1999.

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The nervous system is very sensitive to environmental disturbance. In the proceedings of an international symposium on the "Biological Effects and Health Hazard of Microwave Radiation" hold in Warsaw, Poland in 1973, it was stated in a summary section that 'the reaction of the central nervous system to microwaves may serve as an early indicator of disturbances in regulatory functions of many systems' [Czerski et al., 1974].

Disturbance to the nervous system leads to behavioral changes. On the other hand, alteration in behavior would imply a change in function of the nervous system. Studies on the effect of radiofrequency radiation (RFR) on behavior have been carried out since the beginning of Bioelectromagnetics research. Some of these studies are briefly reviewed below.

It has been speculated that a pulsed RFR is more potent than its continuous-wave (CW) counterpart in causing biological effects [e.g., Barenski, 1972; Frey et al., 1975; Oscar and Hawkins, 1977]. To evaluate this, it is necessary to compare the effects of pulsed RFR with those of CW radiation. Thus, studies on both CW and pulsed (and frequency-modulated) RFRs are included in this review. Comparing the effects of CW and pulsed RFR can actually be related to the popular debate on the distinction between 'thermal' and 'non-thermal/athermal' effect. If an effect is elicited by a pulsed RFR but not by a CW RFR of the same frequency and intensity under the same exposure conditions, it may imply the existence of 'non-thermal/athermal' effect.

Behavior is generally divided into two main categories: spontaneous and learned. Effects of RFR exposure on both types of behavior have been investigated.

Spontaneous Behavior

Spontaneous behaviors are generally considered to be more resistant to disturbance. The most well studied spontaneous behavior in Bioelectromagnetics research is motor (locomotor) activity. Change in motor activity is generally regarded as an indication of change in the arousal state of an animal.

Hunt et al. [1975] reported decreased motor activity in rats after 30 min of exposure to pulsed 2450-MHz RFR (2.5 msec pulses, 120 pps, SAR 6.3 $W \cdot kg^{-1}$). Mitchell et al. [1988] also observed a decrease in motor activity in rats after 7 hr of exposure to CW 2450-MHz RFR (10 $mW{\cdot}cm^{-2}$, average SAR 2.7 $W{\cdot}kg^{-1}$).

Roberti [1975] reported no significant change in locomotor activity in rats after long-term (185-408 h) exposure to RFR of different frequencies (10.7-GHz CW; 3-GHz CW; 3-GHz with 1.3 ms pulses and 770 pps) and various intensities (SAR 0.15-7.5 $W{\cdot}kg^{-1}$). Mitchell et al. [1977] reported an increase in motor activity on a small platform of rats exposed to 2450-MHz RFR (CW, average SAR 2.3 $W{\cdot}kg^{-1}$, 5 hr/day, 5 days/week for 22 weeks). Motor activity of the RFR exposed rats increased during the first week of exposure and stayed higher than controls throughout the period of the experiment. D'Andrea et al. [1979, 1980] reported decreased motor activity on a stabilimetric platform and no significant change in running wheel activity measured overnight in rats exposed to a 2450-MHz RFR (CW, 5 $mW{\cdot}cm^{-2}$, SAR 1.2 $W{\cdot}kg^{-1}$, exposed 5 day/week with a total exposure time of 640 hrs, activity was measured every 2-weeks). However, they reported no significant effect in both behaviors in rats similarly exposed to a 915-MHz RFR even at a higher energy absorption rate (CW, 5 $mW{\cdot}cm^{-2}$, SAR 2.5 $W{\cdot}kg^{-1}$). Moe et al. [1976] reported a decrease in motor activity of rats exposed to 918 MHz RFR (CW, SAR 3.6-4.2 $W{\cdot}kg^{-1}$) during the dark period of the light-dark cycle in a chronic exposure experiment (10 hr/night for 3 weeks). Lovely et al. [1977] repeated the experiment using a lower intensity (2.5 $mW{\cdot}cm^{-2}$, SAR 0.9 $W{\cdot}kg^{-1}$, 10 hr/night, 13 weeks) and found no significant change in motor activity in the exposed rats. Thus, the threshold of response under their exposure conditions is between1 and 4 $W{\cdot}kg^{-1}$.

The results from the above studies indicate that it would need a rather high energy absorption rate (>1 $W{\cdot}kg^{-1}$) to affect motor activity in animals. However, there are two studies reporting effects on motor activity at relatively low SARs. In a long-term exposure study, Johnson et al. [1983] exposed rats to pulsed 2450-MHz RFR (10 ms pulses, 800 pps) from 8 weeks to 25 months of age (22 hr/day). The average whole body SAR varied as the weight of the rats increased and was between 0.4-0.15 $W{\cdot}kg^{-1}$. Open field activity was measured in 3-min sessions with an electronic open-field apparatus once every 6 weeks during the first 15 months and at 12-week intervals in the final 10 weeks of exposure. They reported a significantly lower open field activity only at the first test session, and a rise in the blood corticosterone level was also observed at that time. The authors speculated that RFR might be ‘minimally stressful’ to the rats. Rudnev et al. [1978] studied the behavior of rats exposed to CW 2375-MHz RFR at 0.5 $mW{\cdot}cm^{-2}$ (SAR 0.1 $W{\cdot}kg^{-1}$), 7 h/day for 1 month. They reported a decrease in balancing time in a treadmill and inclined rod and motor activity in an open-field after 20 days of exposure. The open-field motor activity was found to be increased at 3 months post-exposure. Interestingly, Frey [1977] also reported a decrease in motor coordination on a motor-rod in rats exposed to a 1300-MHz pulsed RFR (0.5 ms pulses, 1000 pps, average power density of 0.65 or 0.2 $mW{\cdot}cm^{-2}$).

Another type of spontaneous behavior studied was consummatory behavior. In the Rudnev et al. [1978] study, the authors reported a decrease in food intake in their animals after long-term exposure to CW RFR at 0.1 $W{\cdot}kg^{-1}$. Ray and Behari [1990] also reported a decrease in eating and drinking behavior in rats exposed for 60 days (3 hr/day) to a 7.5-GHz RFR (10-KHz square wave modulation) at an SAR of 0.0317 $W{\cdot}kg^{-1}$ (average power density 0.6 $mW{\cdot}cm^{-2}$).

Learned behavior

Several psychological studies have been carried out to investigate whether animals can detect RFR. One of the early studies was that of King et al. [1971] in which RFR was used as the cue in a conditioned suppression experiment. In conditioned suppression, an animal is first trained to elicit a certain response (e.g., bar-press for food). Once a steady rate of response is attained, a stimulus (e.g., a tone) will be presented to signify the on coming of a negative reinforcement (e.g., electric foot shock). The animal will soon learn the significance of the stimulus and a decrease in responding (conditioned suppression) will occur immediately after the presentation of the stimulus. In the experiment of King et al. [1971], rats were trained to respond at a fixed-ratio schedule for sugar water reward. In a 2-hr session, either a tone or RFR would be presented and occasionally followed by an electric foot shock. Radiofrequency radiation of 2450 MHz, modulated at 12 and 60 Hz and at SARs of 0.6, 1.2, 2.4, 4.8, and 6.4 $W\cdot kg^{-1}$ was used as the conditioned stimulus. With training, consistent conditioned suppression was observed with the radiation at 2.4 $W\cdot kg^{-1}$ and higher. This indicates that rats can detect RFR at 2.4 $W\cdot kg^{-1}$. Monahan and Henton [1977] also demonstrated that mice could be trained to elicit a response in order to escape or avoid RFR (CW, 2450-MHz, 40 $W\cdot kg^{-1}$). In another experiment, Carroll et al. [1980] showed that rats did not learn to go to a 'safe' area in the exposure cage in order to escape exposure to RFR (918-MHz, pulse modulated at 60 Hz, SAR 60 $W\cdot kg^{-1}$) (i.e., entering the 'safe' area resulted in an immediate reduction of the intensity of the radiation), whereas the animals learned readily to escape from electric foot shock by going to the 'safe' area. In a further study from the same laboratory, Levinson et al. [1982] showed that rats could learn to enter a 'safe' area, when the RFR was paired with a light stimulus. Entering the area would turn off both the radiation and light. They also showed that rats could learn to escape by entering the 'safe' area when RFR was presented alone, but learned at a lower rate than when the RFR was paired with a light. All these studies indicate that animals can detect RFR, probably as a thermal stimulus.

One of the most well established effects of pulsed RFR is the 'auditory effect'. Neurophysiological and psychological experiments indicate that animals can probably perceive microwave pulses as a sound stimulus [Chou et al., 1982a; Lin, 1978]. In a series of experiments, Frey and his associates [Frey and Feld, 1975; Frey et al., 1975] demonstrated that rats spent less time in the unshielded compartment of a shuttlebox, when the box was exposed to 1200-MHz pulsed RFR (0.5-ms pulses, 1000 pps, average power density 0.2 $mW\cdot cm^{-2}$, peak power density 2.1 $mW\cdot cm^{-2}$) than during sham exposure. When a CW RFR (1200-MHz, 2.4 $mW\cdot cm^{-2}$) was used, rats showed no significant preference to remain in the shielded or unshielded side of the box. Hjeresen et al. [1979] replicated this finding using pulsed 2880-MHz RFR (2.3 ms pulses, 100 pps, average power density 9.5 $mW\cdot cm^{-2}$) and showed that the preference to remain in the shielded side of a shuttlebox during RFR exposure could be generalized to a 37.5-kHz tone. Masking the 'radiation-induced auditory effect' with a 10-20 kHz noise also prevented shuttlebox-side preference during pulsed RFR exposure. These data indicate that the pulsed RFR-induced 'avoidance' behavior is due to the auditory effect.

The question is why rats avoid pulsed RFR? Is the 'auditory effect' stressful? This question was recently raised by Sienkiewicz [1999]. In an attempt to replicate our radial-arm experiment (Lai et al., 1989), he exposed mice to 900-MHz radiation pulsed at 217 Hz for 45 min a day for 10 days at a whole body SAR of 0.05 $W\cdot kg^{-1}$. He didn't observe any significant effect of RFR exposure on maze learning, but reported that 'some of the exposed animals in our experiment appeared to show a stress-like response during testing in the maze. The animals tested immediately after exposure showed a more erratic performance, and were slower to complete the task compared to the animals tested after a short delay following exposure. This pattern of behavior may be consistent with increased levels of stress.' He also reported that exposed animals showed increased urination and defecation. He speculated that these behavioral effects were caused by the ‘auditory effect’ of the pulsed RFR.

Many studies investigated the effects of RFR exposure on schedule-controlled behavior. A schedule is the scheme by which an animal is rewarded (reinforced) for carrying out a certain behavior. For example, an animal can be reinforced for every response it makes, or reinforced intermittently upon responding according to a certain schedule (e.g., once every ten responses). Schedules of different complexity are used in psychological research. The advantage of using reinforcement schedules is that they generate in animals an orderly and reproducible behavioral pattern that can be maintained over a long period of time. This allows a systematic study of the effect of RFR. Generally speaking, more complex behaviors are more susceptible to disruption by environmental factors. However, the underlying neural mechanisms by which different schedules affect behavior are poorly understood.

In a study by D'Andrea et al. [1977], RFRs of different frequencies and intensities were studied on their effects on bar-pressing rate on a variable-interval schedule. It was found that the latency time of stoppage to respond after the radiation was turned on correlated with the rate of rise in body temperature of the animal. Lebovitz [1980] also studied the effects of pulsed 1300-MHz RFR (1 ms pulses, 600 pps) on rats bar-pressing on a fixed-ratio schedule for food reinforcement. A 15-minute ‘rewarded’ period, when bar pressing was rewarded with food, was followed by a 10-min ‘unrewarded’ period. Both food reinforced bar presses and unrewarded bar presses during the periods were studied. No significant effect was detected in both types of response at SAR of 1.5 $W{\cdot}kg^{-1}$. However, at 6 $W{\cdot}kg^{-1}$, there was a slight reduction in rewarded bar presses and a large reduction in unrewarded bar presses. The authors concluded that the unrewarded behavior was more susceptible to the effect of RFR than the rewarded behavior. However, Hunt et al. [1975] trained rats to bar press for saccharin water rewards in the presence (5- second duration) of a flashing light and not to respond in the presence of a tone. After 30 min of exposure to 2450-MHz RFR (modulated at 20 Hz, SAR of 6.5 or 11.0 $W{\cdot}kg^{-1}$), rats made more misses at the presence of the light, but there were no significant changes in the incidences of bar-pressing error when the tone was on (unrewarded). Gage [1979] trained rats to alternate responses between 2 levers at 11-30 times for a food reinforcement. Decrement in response rates was observed after 15 hrs of exposure to CW 2450-MHz RFR at 10, 15, and 20 $mW{\cdot}cm^{-2}$ (0.3 $W{\cdot}kg^{-1}$ per $mW{\cdot}cm^{-2}$).

Effects of RFR on more complex operant response sequence and reinforcement schedules were studied in various experiments. de Lorge and Ezell [1980] tested rats on an auditory vigilance (observing-response) behavioral task during exposure to pulsed 5620-MHz (0.5 or 2 ms, 662 pps) and 1280-MHz (3 ms, 370 pps) RFR. In this task, rats had to discriminate two tones in order to press one of two bars appropriately for food reinforcement. The task required continuous sensory-motor activities in which the animal had to coordinate its motor responses according to the stimulus cues (tone) presented. Behavioral decrement was observed at a SAR of 3.75 $W{\cdot}kg^{-1}$ with the 1280-MHz radiation, and at 4.9 $W{\cdot}kg^{-1}$ with the 5620-MHz radiation. The authors concluded that ‘…the rat’s observing behavior is disrupted at a lower power density at 1.28 than at 5.62 GHz because of deeper penetration of energy at the lower frequency, and because of frequency-dependent differences in anatomic distribution of the absorbed microwave energy.’ In another experiment, de Lorge [1984] studied rhesus monkeys trained on the auditory vigilance (observing-response) task. After the training, the effects of exposure to RFR of different frequencies (225, 1300, and 5800 MHz) were studied [225-MHz-CW; 1300-MHz- 3 ms pulses, 370 pps; 5800-MHz- 0.5 or 2 ms pulses, 662 pps]. Reduction in performance was observed at different power density thresholds for the frequencies studied: 8.1 mW·cm^{-2} (SAR 3.2 W·kg^{-1}) for 225 MHz, 57 mW·cm^{-2} (SAR 7.4 W·kg^{-1}) for 1300 MHz, and 140 mW·cm^{-2} (SAR 4.3 W·kg^{-1}) for 5800 MHz. de Lorge concluded that the behavioral disruption under different frequencies of exposure was more correlated with change in body temperature. Disruption occurred when the colonic temperature of the animal had increased by 1^{o}C.

Thomas et al. [1975] trained rats to bar press on two bars: a fixed ratio of 20 on the right bar (20 bar presses produced a food pellet reward) and differential reinforcement of low rate (DRL) on the left bar (bar presses had to be separated by at least 18 sec and no more than 24 sec to produce a reward). There was a time-out period between schedules, i.e., no reinforcement available for responding. Animals were tested 5-10 min after 30 min of exposure to either CW 2450-MHz, pulsed 2860-MHz (1 ms pulses, 500 pps) or pulsed 9600-MHz (1 ms pulses, 500 pps) RFR at various power densities. An increase in DRL response rate was observed with 2450-MHz radiation >7.5 mW·cm^{-2} (SAR 2.0 W·kg^{-1}), 2860-MHz RFR >10 mW·cm^{-2} (2.7 W·kg^{-1}), and 9600-MHz RFR >5 mW·cm^{-2} (SAR 1.5 W·kg^{-1}). A decrease in the rate of response at the fixed ratio schedule was seen in all three frequencies when the power density was greater than 5 mW·cm^{-2}. In addition, an increase in response rate was observed during time-out periods under irradiation of the three frequencies of RFR at greater than 5 mW·cm^{-2}. This indicates a disruption of the animals' ability to discriminate the different schedule situations.

Schrot et al. [1980] trained rats to learn a new daily sequence of pressing of three bars for food reinforcement. An increased number of errors and decreased learning rates were observed in the animals after 30 min of exposure to pulsed 2800-MHz RFR (2 ms pulses, 500 pps) at average power densities of 5 and 10 mW·cm^{-2} (SAR 0.7 and 1.7 W·kg^{-1}, respectively). No significant effect on performance was observed at power densities of 0.25, 0.5, and 1 mW·cm^{-2}.

D'Andrea et al. [1989] studied the behavioral effects of high peak power RFR pulses of 1360-MHz. Rhesus monkeys performing on a complicated reinforcement-schedule involving time-related behavioral tasks (inter-response time, time discrimination, and fixed interval responses) were exposed to high peak power RFR (131.8 W·cm^{-2} rms, pulse repetition rate 2-32 Hz). No significant disturbance in performance was observed in the monkeys. Akyel et al. [1991] also studied the effects of exposure to high peak power RFR pulses on behavior. In their experiment, rats pre-trained to bar-press for food reinforcement on either fixed ratio, variable interval, or DRL schedule were exposed for 10 min to 1250-MHz pulses. Each pulse (10 ms width) generated a whole body specific absorption of 2.1 J·kg^{-1}, which corresponds to a whole body average SAR of 0.21 mW·kg^{-1}. The pulse rate was adjusted to produce different total doses (0.5-14 kJ·kg^{-1}). Only at the highest dose (14 kJ·kg^{-1}), stoppage of responding was observed after exposure, when the colonic temperature was increased by ~2.5^{o}C. Responding resumed when colonic temperature returned to within 1.1^{o}C above the pre-exposure level. When responding resumed, the response rates on the fixed ratio and variable interval schedules were below the pre-exposure base line level. Responses on the DRL schedule were too variable to allow a conclusion to be drawn. The authors concluded that the effect of the high peak power RFR pulses on schedule-controlled behavior was due to hyperthermia.

Several studies investigated the effects of long-term RFR exposure on schedule controlled-behavior. Mitchell et al. [1977] trained rats to respond on a mixed schedule of reinforcement (FR-5 EXT-15 sec), in which 5 responses would give a reward and then a 15 sec lapse time (extinction period) was required before a new response would be rewarded. In addition, the schedule of reinforcement was effective when a lamp was on, while no reinforcement was given when the lamp was off. Rats were then exposed to CW 2450-MHz

RFR (average SAR 2.3 W·kg^{-1}) for 22 weeks (5 hr/day, 5 days/week) and tested at different times during the exposure period. The RFR-exposed rats showed higher responses during the extinction period, indicating poorer discrimination of the response cues. Navakatikian and Tomashevskaya [1994] described a complex series of experiments in which they observed disruption of a behavior (active avoidance) by RFR. In the study, rats were first trained to perform the behavior and then exposed to either CW 2450-MHz RFR or pulsed 3000-MHz RFR (400-Hz modulation, pulse duration 2 ms, and simulation of radar rotation of 3, 6, and 29 rotations/min) for 0.5-12 hrs or 15-80 days (7-12 hr/day). Behavioral disruption was observed at a power density as low as 0.1 mW·cm^{-2} (0.027 W·kg^{-1}).

Two series of well-designed experiments were run by D'Andrea and his colleagues to investigate the effects of chronic RFR exposure on behavior. In one experiment [D'Andrea et al., 1986 a], rats were exposed for 14 weeks (7 hr/day, 7 days/week) to CW 2450-MHz RFR at 2.5 mW·cm^{-2} (SAR 0.7 W·kg^{-1}). After exposure, the rats were trained to bar press on an interresponse time criterion (IRT). In this schedule, the animals had to respond within 12 to 18 sec after the previous response in order to receive a food reward. Radiofrequency radiation exposed rats emitted more responses during the training period. When the training was completed, the RFR-exposed rats had lower efficiency in bar-pressing to obtain food pellets, i.e., they made more inappropriate responses and received fewer food pellets than the sham-exposed rats during a session. In a signalled two-way active avoidance shuttlebox test, the RFR-exposed rats showed less avoidance response than the sham-exposed rats during training; however, no significant difference in responses in the shuttlebox test was detected at 60 days after exposure between the RFR- and sham-exposed animals. In this experiment, a decrease in the threshold of electric foot shock detection (i.e., increase in sensitivity) was also observed in the irradiated rats during the exposure period, and an increased open-field exploratory behavior was observed in the rats at 30 days post-exposure. It may be interesting to point out that Frey [1977] also reported a decrease in tail pinch-induced aggressive behavior in RFR-exposed rats. Increased latency, decrease in duration, and episodes of fighting after tail pinching were observed between two rats being irradiated with RFR. This could be due to a decreased sensitivity or perception of pain and the RFR-induced activation of endogenous opioids described below.

In a second experiment [D'Andrea et al., 1986 b], rats were exposed to 2450-MHz RFR at 0.5 mW·cm^{-2} (SAR 0.14 W·kg^{-1}) for 90 days (7 hr/day, 7 days/week). Open-field behavior, shuttlebox performance, and schedule-controlled bar-pressing behavior for food pellets were studied at the end of the exposure period. A small deficit in shuttlebox performance and an increased rate of bar-pressing were observed in the RFR exposed rats. Summarizing the data from these two series of experiments [D'Andrea et al., 1986 a,b], D'Andrea and his co-workers concluded that the threshold for the behavioral and physiological effects of chronic RFR exposure in the rats studied in their experiments occurred between the power densities of 0.5 mW·cm^{-2} (SAR 0.14 W·kg^{-1}) and 2.5 mW·cm^{-2} (SAR 0.7 W·kg^{-1}).

In a further experiment, DeWitt et al. [1987] also reported an effect on an operant task in rats after exposure for 7hr/day for 90 days to CW 2450-MHz RFR at a power density of 0.5 mW·cm^{-2} (0.14 W·kg^{-1}).

Little work has been done to investigate the effects of RFR on memory functions. We [Lai et al., 1989] studied the effect of short-term (45 min) RFR exposure (2450-MHz, 2 msec pulses, 500 pps, 1 mW·cm^{-2}, SAR 0.6 W·kg^{-1}) on the rats' performance in a radial-arm maze, which measures spatial working (short-term) memory function. The maze consists of a central circular hub with arms radiating out like the spokes of a wheel. In this task, food-deprived animals are trained to explore the arms of the maze to obtain food reinforcement at the end of each arm. In each session they have to enter each arm once and a reentry is considered as an error. This task requires 'working memory', i.e., the rat has to remember the arms it has already entered during the course of a session. We found that short-term (45 min) exposure to RFR before each session of maze running significantly retarded the rats' abilities to perform in the maze. They made significantly more errors than the sham-exposed rats. In a further experiment [Lai et al., 1994], we found that the RFR-induced working memory deficit in the radial-arm maze was reversed by pretreating the rats before exposure with the cholinergic agonist physostigmine or the opiate antagonist naltrexone, whereas pretreatment with the peripheral opiate antagonist naloxone methiodide showed no reversal of effect. These data indicate that both cholinergic and endogenous opioid neurotransmitter systems inside the central nervous system are involved in the RFR-induced spatial working memory deficit. Spatial working memory requires the functions of the cholinergic innervations in the frontal cortex and hippocampus. The behavior result agrees with our previous neurochemical findings that RFR exposure decreased the activity of the cholinergic systems in the frontal cortex and hippocampus of the rats [Lai et al., 1987]. Endogenous opioids [Lai et al., 1992] and the ‘stress hormone’ corticotropin-releasing factor [Lai et al., 1990] are also involved. Our hypothesis is that radiofrequency radiation activates endogenous opioids in the brain, which in turn cause a decrease in cholinergic activity leading to short-term memory deficit. Related to this that there is a report by Kunjilwar and Behari [1993] showing that long-term exposure (30-35 days, 3 hrs/day, SAR 0.1-0.14 W/kg) to 147-MHz RFR and its sub-harmonics 73.5 and 36.75 MHz, amplitude modulated at 16 and 76 Hz, decreased acetylcholine esterase activity in the rat brain, whereas short-term exposure (60 min) had no significant effect on the enzyme. There is another report by Krylova et al. [1992] indicating that ‘cholinergic system plays an important role in the effects of electromagnetic field on memory processes’. There are also two studies suggesting the involvement of endogenous opioids in the effects of RFR on memory functions [Krylov et al., 1993; Mickley and Cobb, 1998].

In a more recent experiment, we [Wang and Lai, 2000] studied spatial long-term memory using the water maze. In this test, rats are trained to learn the location of a submerged platform in a circular water pool. We found that rats exposed to pulsed 2450-MHz RFR (2 ms pulses, 500 pps, 1.2 $W{\cdot}kg^{-1}$, 1 hr) were significantly slower in learning and used a different strategy in locating the position of the platform.

Comments

(1) From the data available, it is not apparent that pulsed RFR is more potent than CW RFR in affecting behavior in animals. Even though different frequencies and exposure conditions were used in different studies and hardly any dose-response study was carried out, there is no consistent pattern that the SARs of pulsed RFR reported to cause an effect are lower than those of CW RFR. For example, the Thomas et al [1975] study showed that the thresholds of effect of CW 2450-MHz (2.0 $W{\cdot}kg^{-1}$) and pulsed 2860-MHz (2.7 $W{\cdot}kg^{-1}$) radiation on DRL bar-pressing response are quite similar.

(2) Thermal effect is definitely a factor in the effects reported in some of the experiments described above. A related point is that most psychoactive drugs also affect body temperature. Stimulants cause hyperthermia, barbiturates cause hypothermia, and narcotics have a biphasic effect on body temperature (hyperthermia at low doses and hypothermia at high doses). It is not uncommon to

observe a change of 2-3°C within 30 min after a drug is administered. However, in reviewing the literature, there is no general correlation between the effects of psychoactive drugs on body temperature and schedule-controlled behavior. Thus, body temperature may not be a major factor in an animal's responding under schedule-controlled behavior, at least in the case of psychoactive drugs. On the contrary, some of the experiments described above strongly suggest the role of hyperthermia on the RFR effect on the behavior. Perhaps, a sudden and large increase in body temperature as in the case of RFR can have a major effect on responding.

(3) Generally speaking, when effects were observed, RFR disrupted schedule-controlled behavior in animals such as in the cases of discrimination responding [de Lorge and Ezell, 1980; Hunt et al., 1975; Mitchell et al., 1977], learning [Schrot et al., 1980], and avoidance [D'Andrea et al., 1986 a,b]. This is especially true when the task involved complex schedules and response sequence. In no case has an improvement in behavior been reported in animals after RFR exposure. It is puzzling that only disruptions in behavior by RFR exposure are reported. In the studies on EEG, both excitation (desynchronization) and depression (synchronization) have been reported after exposure to RFR [Bawin et al., 1973; Chizhenkova, 1988; Chou et al., 1982b; Dumansky and Shandala, 1974; Goldstein and Sisko, 1974; Takeshima et al., 1979]. Motor activity has also been reported to increase [D'Andrea et al., 1979, 1980; Frey et al., 1975; Hjeresen et al., 1979; Mitchell et al., 1977; Rudnev et al., 1978] and decrease [Hunt et al., 1975; Johnson et al., 1983; Mitchell et al., 1988; Moe et al., 1976; Rudnev et al., 1978] after RFR exposure. If these measurements can be considered as indications of electrophysiological and behavioral arousal and depression, improvement in behavior should occur under certain conditions of RFR exposure. This is especially true with avoidance behavior. Psychomotor stimulants that cause EEG desynchronization and motor activation improve avoidance behavior, whereas tranquilizers that have opposite effects on EEG and motor activity decrease avoidance behavior.

(4) It is difficult to conclude from the effects of RFR on schedule-controlled behavior the underlying neural mechanisms involved. In general, the effects of the effect of RFR on schedule-controlled behavior is similar to those of other agents, e.g., psychoactive drugs. For example, the way that a certain drug affects schedule-controlled behavior depends on the base line level of responding. A general rule is that drugs tend to decrease the rate when the base line responding rate is high and vice versa. This is known as rate-dependency. Exposure to RFR caused a decrease in response rate when a variable interval schedule that produces a steady rate of responding was used [D'Andrea et al., 1976; 1977], and an increase in responding when the DRL-schedule of reinforcement, that produces a low base line of responding, was used [Thomas et al., 1975]. This may reflect a rate-dependency effect. The effect of an agent can also depend on the schedule of reinforcement. For example, amphetamine has different effects on responses maintained on DRL schedule and punishment-suppressed responding schedule, even though both schedules generate a similar low response rate. Stimulus control as a determinant of response outcome was seen in the study of Lebovitz [1980] when unrewarded responses were disrupted more by RFR than rewarded responses, and the study of Hunt et al. [1975] that showed the reverse relationship. In the former experiment a fixed interval schedule was used, whereas in the latter a discrimination paradigm was studied.

(5) It is also interesting to point out that in most of the behavioral experiments, effects were observed after the termination of RFR exposure. In some experiments (e.g., Rudnev et al., 1978; D'Andrea et al., 1986 a,b), tests were made days after exposure. This suggests a persistent change in the nervous system after exposure to RFR.

(6) In many instances, effects on learned behavior were observed at a SAR less than 4 $W{\cdot}kg^{-1}$. (D'Andrea et al [1986a,b] 0.14 to 0.7 $W{\cdot}kg^{-1}$; DeWitt et al. [1987] 0.14 $W{\cdot}kg^{-1}$; Gage [1979] 3 $W{\cdot}kg^{-1}$; King et al.[1971] 2.4 $W{\cdot}kg^{-1}$; Lai et al. [1989] 0.6 $W{\cdot}kg^{-1}$; Mitchell et al. [1977] 2.3 $W{\cdot}kg^{-1}$; Navakatikian and Tomashevskaya [1994] 0.027 $W{\cdot}kg^{-1}$; Schrot et al. [1980] 0.7 $W{\cdot}kg^{-1}$; Thomas et al. [1975] 1.5 to 2.7 $W{\cdot}kg^{-1}$; Wang and Lai [2000] 1.2 $W{\cdot}kg^{-1}$).

(7) Does disturbance in behavior have any relevance to health? The consequence of a behavioral deficit is situation dependent and may not be direct. It probably does not matter if a person is playing chess and RFR in his environment causes him to make a couple of bad moves. However, the consequence would be much more serious if a person is flying an airplane and his response sequences are disrupted by RFR radiation.

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SECTION 9

Neurological Effects of Non-Ionizing Electromagnetic Fields

2014 Supplement

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I. INTRODUCTION

Neurological effects are caused by changes in the nervous system. Factors that act directly or indirectly on the nervous system causing morphological, chemical, or electrical changes in the nervous system can lead to neurological effects. The final manifestation of these effects can be seen in psychological changes, e.g., memory, learning and perception. The nervous system is an electrical organ. Thus, it should not be surprising that exposure to electromagnetic fields could lead to neurological changes. Morphological, chemical, electrical, and behavioral changes have been reported in animals and cells after exposure to nonionizing electromagnetic fields (EMF) across a range of frequencies. The consequences of physiological changes in the nervous system are very difficult to assess. We don't quite understand how the nervous system functions and reacts to external perturbations. The highly flexible nervous system could easily compensate for external disturbances. On the other hand, the consequence of neural perturbation is also situation-dependent. An EMF-induced change in brain electrical activity, for instance, could lead to different consequences depending on whether a person is watching TV or driving a car.

The following is a summary of the research literature on the neurological effects of EMF exposure published between 2007-2014. The literature on radiofrequency and extremely-low frequency EMFs are placed in two separate sections. Each section has a discussion and a list of publications with abstracts. Summary sentences in the abstracts are underlined for reader convenience. Where additional information is relevant, some earlier papers, or papers not specifically related to neurological effects, are also included with citations contained within the discussion.

In this paper, as in the update paper on genetic effects, analyses show that there are more publications showing effects than no effects with the recent neurological literature. With E representing a biological effect, and NE representing no biological effects, the recent literature finds **in 211 studies**, **RFR-neurological effects at: E=144 publications (68%); NE=67 publications (32%); and 105 ELF-neurological effects studies: E=95 (90%); NE=10 (10%).**

Appendix A has references and abstracts for the RFR literature.
Appendix B has references and abstracts for the ELF-EMF literature.

II. NEUROLOGICAL EFFECTS OF RADIOFREQUENCY RADIATION (RFR) - (2007-2014)

Discussion

(1) There are many new studies on human subjects. Many of them are on changes in brain electrical activities after acute exposure to cell phone radiation. Bak et al (2010) reported effects on event-related potentials. Maganioti et al. (2010) further reported that RFR affected the gender-specific components of event-related potentials (see also Hountala et al., 2008). Croft et al (2008) reported changes of the alpha-wave power of EEG. The same authors (Croft et al., 2010) further reported that effects differed between various new cell phone transmission systems, which have different signaling characteristics. They observed effects after exposure to second generation (2G), but not third generation (3G) radiation, whereas Leung et al. (2011) found similar EEG effects with both 2G and 3G radiations. Lustenberger et al. (2013) found increased slow-wave activity in humans during exposure to pulse-modulated RF EMF toward the end of the sleep period. Vecchio and associates reported that cell phone RFR affected EEG and the spread of neural synchronization conveyed by interhemispherical functional coupling of EEG rhythms (Vecchio et al., 2007) and enhanced human cortical neural efficiency (Vecchio et al., 2012a). An interesting finding is that RFR could interact with the activity of brain epileptic foci in epileptic patients (Tombini et al., 2012; Vecchio et al., 2012b). However, no significant effect on EEG was reported by Parentos et al. (2007) or Trunk et al. (2012), and Kleinlogel et al. (2008 a, b) also reported no significant effects on resting EEG and event-related potentials in humans after exposure to cell phone RFR. Furthermore, Krause et al. (2007) reported no significant effect of cell phone radiation on brain oscillatory activity, and Inomata-Terada et al. (2007) concluded that cell phone radiation does not affect the electrical activity of the motor cortex.

(2) There are studies on the interaction of cell phone radiation on EEG during sleep. Changes in sleep EEG have been reported by Hung et al. (2007), Regel et al. (2007), Lowden et al (2011), Schmid et al. (2012), and Loughran et al. (2012), whereas, no significant effect was reported by Fritzer et al (2007), Mohler et al. (2010, 2012) and Nakatani-Enomoto et al. (2013). Loughran et al. (2012) provided an interesting conclusion in their paper: "These results confirm previous findings of mobile phone-like emissions affecting the EEG during non-REM sleep. Importantly, this low-level effect was also shown to be sensitive to individual variability. Furthermore, this indicates that "previous negative results are not strong evidence for a lack of an effect…" Increase in REM sleep was reported by Pelletier et al. (2012) in developing rats after chronic exposure. Mohammed et al. (2013) reported a disturbance in REM sleep EEG in the rat after long term exposure (1 hr/day for 1 month) to a 900-MHz modulated RFR.

(3) With these electrophysiological changes in the brain, what behavioral effects have been reported? The outcomes are summarized in the tables below. The animal studies are mostly studies on rodents (i.e., rat and mouse).

Human studies that showed behavioral effects:

	Behavior studies/results	**Exposure duration**
de Tommaso et al. (2009)	Reduction in behavioral arousal	10 min
Hung et al. (2007)	Sleep latency	30 min
Leung et al. (2011)	Cognitive functions	10 min
Luria et al. (2009)	Spatial working memory (In a subsequent study (Hareuveny et al., 2011), the authors indicated that some of the effects observed may not be related to RFR exposure.)	60 min
Lustenberger et al. (2013)	Sleep-dependent motor-task performance improvement	All-night
Redmayne et al. (2013)	Well-being	Use of cellphone and cordless phone
Regel et al. (2007)	Cognitive functions	30 min
Thomas et al. (2010b)	Overall behavioral problems in adolescents	
Vecchio et al. (2012b)	Enhanced cognitive-motor processes	45 min
Vecsei et al. (2013)	Thermal pain threshold	30 min
Wiholm et al. (2009)	‘Virtual’ spatial navigation task	150 min

Human studies that did not show behavioral effects:

	Behavior studies/results	**Exposure duration**
Cinel et al. (2007)	Order threshold task	40 min
Cinel et al. (2008)	Subjective symptoms	40 min
Curcio et al. (2008)	Reaction time task, sequential figure tapping task	3 x 15 min

Curcio et al. (2012)	Somatosensory task	40 min
Danker-Hopfe et al. (2011)	Effect on sleep	
Eltiti et al. (2009)	Cognitive functions	50 min
Fritzer et al. (2007)	Sleep and cognitive functions	During sleep
Haarala et al. (2007)	Cognitive functions	90 min
Irlenbusch et al. (2007)	Visual discrimination threshold	30 min
Kleinlogel et al. (2008a)	Well being	30 min
Loughran et al. (2013)	Cognitive effects and EEG	30-60 min
Mohler et al. (2010, 2012)	Effect on sleep	
Nakatani-Enomoto et al. (2013)	Effect on sleep	3 hr
Riddervold et al. (2008)	Trail making B test	45 min
Sauter et al. (2011)	Cognitive functions	7 hr 15 min in two episodes
Schmid et al. (2012a)	Cognitive functions	30 min
Schmid et al. (2012b)	Cognitive functions	30 min
Unterlechner et al. (2008)	attention	90 min
Wallace et al. (2012)	Cognitive functions	10- 50 min (whole body exposure)

Animal studies that showed behavioral effects:

	Behavior studies/results	**Exposure duration**
Aldad et al. (2012)	Hyperactive, impaired memory	In utero
Arendash et al. (2010, 2012)	Improved cognitive behavior	Daily, 2-6 months
Bouji et al. (2012)	Contextual emotional behavior deficit	15 min
Cammaerts et al. (2012)	Olfactory and/or visual	

	memory deficit in ants	
Cammaerts et al. (2013)	Food collection behavior of ants	180 hr
Daniels et al. (2009)	Decreased motor activity	
Deshmukh et al. (2013)	Cognitive functions	2 hr/day, 30 days
Fragopoulou et al. (2010)	Spatial memory deficit	2 hr/day, 4 days
Hao et al. (2012)	Learning and memory deficit	6 hr/day, 5 days/wk, 10 wk
İkinci et al. (2013)	Learning behavior deficit	Prenatal exposure
Júnior et al. (2014)	Stress behavioral patterns	25 sec every 2 min for 3 days
Kumar et al. (2009)	hypoactivity	50 missed call/day, 4 wk
Kumlin et al. (2007)	Improved learning and memory	2 hr/day. 5 days/wk, 5 wk
Lu et al. (2012)	Spatial memory deficit	3 hr/day, 30 days
Maaroufi et al. (2013)	Spatial learning and memory deficit	1 hr/day, 21 days
Mathur (2008)	Analgesic effect	2 hr/day, 45 days
Megha et al. (2012)	Cognitive functions	2 hr/day, 30 days
Narayanan et al. (2009)	Learning deficit	50 missed call/day, 4 wk
Narayanan et al. (2010)	Passive avoidance deficit	50 missed call/day, 4 wk
Narayanan et al. (2012)	Elevated plus maze-emotionality test	28 days
Nittby et al. (2008)	Reduced memory functions	2 hr/wk, 55 wk
Ntzouni et al. (2011)	Non-spatial memory deficit	90 min/day, 17 days
Ntzouni et al. (2013)	Spatial and non-spatial memory deficit	90 min/day, 66-148 days
Odacı et al. (2013)	Motor function	Prenatal exposure
Pelletier et al. (2012)	Food intake increase	5 weeks
Qin et al. (2014)	Learning and memory deficits	2 hr/day, 30 days

Razavinasab et al. (2014)	Learning and memory deficits	In utero
Sarapultseva et al. (2013)	Motor activity in protozoa	0.05-10 hr
Sharma et al. (2013)	Spatial memory deficit	2 hr/day, 30 days
Sokolovic et al. (2012)	Anxiety-related behavior	4 hr/day for 20, 40, 60 days
Vácha et al. (2009)	Magnetoreception in cockroach	
Wang et al. (2013)	Spatial memory deficit	6 min

Animal studies that did not show behavioral effects:

	Behavior studies/results	**Exposure duration**
Ammari et al. (2008c)	spatial memory	15 min/day, 8 or 24 wk
Haghani et al. (2013)	Motor function	6 hr/day during gestation period

Almost all the animal studies reported effects, whereas more human studies reported no effects than effects. This may be caused by several possible factors: (a) Humans are less susceptible to the effects of RFR than are rodents. (b) It may be more difficult to do human than animal experiments, since it is, in general, easier to control the variables and confounding factors in an animal experiment. (c) In the animal studies, the cumulative exposure duration was generally longer and studies were carried out after exposure, whereas in the human studies, the exposure was generally one time and testing was done during exposure. This raises the question of whether the effects of RFR are cumulative. This consideration could have very important implication on real life human exposure to EMF. However, it must be pointed out that neurophysiological and behavioral changes have been reported in both animals and humans after acute (one time) exposure to RFR, and most of the EEG studies mentioned above are acute exposure experiments. (In the 2007-2013 papers listed below, see those marked '**(E)**' and not classified as '**CE**'). (d) In the animal studies, the effects studies were mostly learning and memory functions. The hippocampus in the brain, particularly the cholinergic system, plays a major role in learning and memory functions. Various studies (2007-2013) indicated that RFR affected the activities/morphology/chemistry of the hippocampus in animals (Aboul Ezz et al., 2013; Ammari et al., 2010; Barcal et al., 2007; Baş et al., 2009, 2013; Carballo-Quintas et al., 2011; Fragopoulous et al., 2012; Hao et al., 2012; İkinci et al., 2013; Kesari et al., 2011; Lopez-Martin et al., 2009; Lu et al., 2012; Maskey et al., 2010 a,b, 2012; Narayanan et al., 2010; Ning et al., 2007; Nittby et al., 2008; Odaci et al., 2008; Razavinasab et al., 2014; Tong et al., 2013; Wang et al., 2013; Yang et al., 2012). (Reports on effects of the hippocampus can also be found in the ELF section below). As early as 1987, we have reported that RFR affected cholinergic system in the hippocampus of the rat (Lai H, Horita A, Chou CK, Guy AW. Low-level microwave irradiation affects central cholinergic activity in the rat. J Neurochem. 48:40-45, 1987). Thus, it is not surprising that 'learning and memory' functions are affected in the rodents by RFR. In the human studies listed above, the most common effect studied was cognitive function. Since the exposure in most of these human studies was localized in the brain, particularly in the temporal cortical area, it is questionable whether the psychological tests used were appropriate.

(4) There are studies on the effects of cell phone radiation and the auditory system. Most research (Kwon 2009, 2010a, b; Parazzini et al., 2009; Stefanics et al., 2007, 2008) reported no effects, which seems to agree with the pre-2007 studies in this area. However, there are two reports by Kaprana et al. (2011) and Khullar et al (2013) showing effects on auditory brainstem response, two papers by Panda et al (2010, 2011) that concluded: "Long-term and intensive GSM and CDMA mobile phone use may cause damage to cochlea as well as the auditory cortex.", and a paper (Mandala et al., 2013) reporting effect on auditory-evoked cohlear nerve response. Maskey et al. (2013) reported chemical changes in the superiou olivary complex, a neural component of the auditory system, in mice after chronic exposure to RFR. Velayutham et al. (2014) reported hearing loss in cell phone users and Sudan et al. (2013) observed weak associations between cell phone use and hearing loss in children at age 7. These effects may not be caused by the radiation.

(5) There are several studies that showed neurological changes in humans after use of wireless devices, but those changes apparently were not caused by exposure to the radiation. Abramson et al. (2009) reported changes in cognitive functions in young adolescents. ("The accuracy of working memory was poorer, reaction time for a simple learning task shorter, associative learning response time shorter and accuracy poorer in children reporting more mobile phone voice calls"). Arns et al. (2007) observed more focused attention in frequent cell phone users, which was probably a "cognitive training effect". Yuan et al. (2011) reported morphological changes in the brain of adolescents with "internet addiction disorder".

(6) There are several studies showing differential effects of different waveforms. This is an important consideration in understanding how EMF interacts with living organisms and nonthermal effects. Croft et al. (2010) reported that 2G, but not 3G, cell phone radiation affected resting EEG. Hung et al. (2007) showed that 2, 8, 217 Hz-modulated RFR differentially affected sleep. Lopez-Martin et al. (2009) reported that modulated and non-modulated RFR had different effects on gene expression in the brain. Nylund et al. (2010) found that different carrier-frequencies (900 MHz verses 1800 MHz) had different effects on protein expression. Schmid et al. (2012) concluded that "modulation frequency components (of a RFR) within a physiological range may be sufficient to induce changes in sleep EEG". Zhang et al. (2008) reported that an intermittent exposure to RFR had a more potent effect on gene expression in the brain than a continuous exposure. Apparently, ELF-modulation plays a role on determining the biological effects of RFR. Indeed, in the following section on the neurological effects of ELF EMF, one can find many studies showing EEG and behavioral effects in animals after exposure to ELF

fields (Capone et al., 2009; Carrubba et al., 2007, 2010; Cook et al., 2009; Corbacio et al., 2011; Cvetkovic and Cosic, 2009; Legros et al., 2012; Perentos et al., 2008; Ross et al., 2008; Shafiei et al., 2012; Shin et al., 2007, 2011; Stevens, 2007). This is of considerable importance, since all cell phone signals are modulated by low frequency components.

(7) In the 2007-2014 literaure below on the neurological effects of RFR, there are several papers indicating that oxidative stress played a role in the effects observed: Cetin et al., 2014; Dasdag et al., 2009, 2012; Del Vecchio et al., 2009; Deshmukh et al., 2013a; Dragicevic et al., 2011; Eser et al., 2013; Gao et al., 2013; Imge et al., 2010; Jing et al., 2012; Kesari et al., 2011; Liu et al., 2011; Maaroufi et al., 2013; Megha et al., 2012; Meral et al., 2007; Nazıroğlu et al., 2012; Qin et al., 2014; Sokolovic et al., 2009; Xu et al., 2010. (Dragicevic et al. (2011) reported a decrease in mitochondrial free radical production in the hippocampus and cerebral cortex of the mouse after RFR exposure.) There was one study (Poulletier de Gannes et al, 2011) that found no significant oxidative stress in brain cells after exposure to Enhanced Data rate for GSM Evolution (EDGE) signal. Kang et al (2013) reported that “neither combined RF radiation alone nor combined RF radiation with menadione or H2O2 influences the intracellular ROS level in neuronal cells.” The mediating roles of cellular free radicals and oxidative status on the biological effects of EMF are worth looking into.

(8) An important issue that has been extensively debated in the media is whether children are more vulnerable to the effect of cell phone radiation than adults? The claim that children have thinner skulls and thus absorb more energy is not valid. And the claim that a child’s head absorbs more energy from a cell phone is also debatable. It is quite possible that the pattern of energy distribution of cell phone energy absorption in the head is significantly different between a child and an adult (cf. Christ A, Kuster N. Differences in RF energy absorption in the heads of adults and children. Bioelectromagnetics. Suppl 7:S31-44. 2005; Christ A, Gosselin MC, Christopoulou M, Kühn S, Kuster N. Age-dependent tissue-specific exposure of cell phone users. Phys. Med. Biol. 55(7):1767-1783, 2010; Gandhi OP, Morgan LL, de Salles AA, Han YY, Herberman RB, Davis DL. Exposure limits: the underestimation of absorbed cell phone radiation, especially in children. Electromagn. Biol. Med. 31(1):34-51, 2012.). Scientific data on whether a child is biologically more vulnerable to cell phone radiation is sparse. In the 2007-2014 literature that I surveyed, there are several studies that indicate that animals (including humans) of different ages respond differently to cell phone radiation. Bouji et al. (2012) reported differences in neuro-immunity, stress, and behavioral responses to GSM signals between ‘young adult’ (6 weeks-old) and ‘middle age’ (12 month-old) rats. Croft et al. (2010) showed that GSM signals affected certain electrical activities of the brain in young human adults (19-40 years old) but not in adolescents (13-15 years old) or elderly (55-70 years old) subjects. Leung et al. (2011) reported that performance in a cognitive test was affected by GSM signal in adolescents but not in young or old human subjects. Noor et al. (2011) reported differences in neurochemical responses to 900-MHz RFR between adult and young rats. And, Vecchio et al. (2010) found differences in brain electric activities between young and elderly human subjects responding to GSM signals. It must be pointed out that although these studies reported an age-dependent effect of cell

phone radiation, they do not necessarily imply that children are more vulnerable to cell phone radiation than adults. (See also: Sekeroğlu V, Akar A, Sekeroğlu ZA. Cytotoxic and genotoxic effects of high-frequency electromagnetic fields (GSM 1800 MHz) on immature and mature rats. Ecotoxicol Environ Saf. 80:140-144, 2012.) There are several papers showing effects of exposure to RFR during perinatal periods on the development and functions of the nervous system (Aldad et al., 2012; Bas et al., 2013; Cetin et al., 2014; Divan et al., 2008, 2011, 2012; Gao et al., 2013; Haghani et al., 2013; İkinci et al., 2013; Jing et al., 2012; Kokturk et al., 2013; Odaci et al., 2008, 2013; Ragbetli e al., 2010; Razavinasab et al., 2014; Zareen et a., 2009). The cerebellum seems to be a structure especially vulnerable to the exposure (Eser et al. 2013; Haghani et al., 2013; Kokturk et al., 2013; Ragbetli e al., 2010).

(9) In many of these studies, a cell phone was used in the exposure of animals and humans. But information on how the cell phone was activated, in many instances, was not provided. Thus, the amount of energy deposited in the body was not known. Some studies used the phone in ‘stand-by’ mode. Kjell Mild and his associates reported that when a stationary cell phone is on ‘stand-by’ mode, it actually infrequently emits a very small amount of energy (Mild KH, Andersen JB, Pedersen GF. Is there any exposure from a mobile phone in stand-by mode? Electromagn Biol Med. 31(1):52-56, 2012).

(10) I think that a few words should be said about ‘thermal’ and ‘nonthermal’ effects. It is not easy to conclude that an RFR effect is ‘nonthermal’, because of the uneven distribution of the energy in the body. On the other hand, it is also not easy to prove that an effect is ‘thermal’. There is an important criterion for the proof of ‘nonthermal’ effect. It is ‘modulation effect’. If you expose an animal or cells at the same frequency and SAR (thus, the same distribution and amount of energy) but at different modulations (i.e., energy is delivered with different time sequences) and produce different effects, then it is good proof of a nonthermal effect. Most studies do not include different modulations. Thus, the effects reported by these studies cannot be concluded as ‘nonthermal’. There are some studies, however, that reported different biological effects with RFRs of the same frequency and intensity but different modulations (see point #6 above and the section on ‘genetic effects’, and some of my earlier papers). From these; I would conclude that nonthermal effects probably exist. Another important argument for EMF nonthermal effects is that low-level ELF-EMF can produce biological effects. The energy carried by ELF-EMF is very small and thermal effect is unlikely. (High intensity ELF-EMF can produce electric currents in the body and possibly heating.) The ‘thermal/nonthermal’ distinction is purely a scientific question. In public exposure policy, we only need to know at what level of exposure an effect occurs. Exposure guideline should be set based on it, and it doesn’t matter whether the effect is thermal or nonthermal.

III. NEUROLOGICAL EFFECTS OF EXTREMELY-LOW FREQUENCY ELECTROMAGNETIC FIELDS (ELF-EMF) (2007-2014)

Discussion

The following is a summary of the research literature on the neurological effects of ELF EMF published in 2007-2014. (In most studies, even only magnetic field was mentioned; there was no explicit statement that electric fields had been eliminated. In most ELF EMF exposure systems used in laboratory system, electric fields were also generated unless grounding was done. Thus, cells or animals were actually exposed to both magnetic and electric fields.)

1. Neurotransmitters are chemicals that carry (transmit) signals from one nerve cell to another. Neurotransmitters are released from one nerve cell and react with molecules called receptors on another nerve cell. The reaction alters the activity of the second nerve cell. Activities in nerve cell could also change the properties of these receptors (mainly by changing the concentration or the affinity of the receptors to neurotransmitters). In the updated EMF literature, all the studies are on the effects of ELF EMF exposure on neurotransmitter receptors. Manikonda et al. (2007) reported effects of chronic ELF EMF exposure on NMDA receptors in the hippocampus of the rat. Salunke et al. (2013) reported that ELF EMF-induced anxiety in the rat involved NMDA receptors in the brain. There is a report on effects of magnetic field serotonin and dopamine receptors in the brain of the rat (Janac et al., 2009). Changes in a subtypes of serotonin receptors 5HT(2A) in the prefrontal cortex was reported. However, Masuda et al. (2011) reported that another types of serotonin receptor 5HT (1B) was not significantly affected after magnetic field exposure in an in vitro experiment. The research were trying to replicate two experiments carried out previously showing magnetic field exposure affected 5HT(1B) receptor. Some of the co-authors of the Musuda study were actually co-authors of one of these earlier studies. However, the 5HT(2A) receptors , particularly in the frontal cortex, are believed to be related to the psychiatric syndromes of depression in humans. Kitaoka et al. (2013) and Szemerszky et al. (2010) did report depression-like behavior in mice and rats, respectively, after chronic exposure to magnetic fields. There are two reports on dopamine receptors. Shin et al. (2007, 2011) reported an increase in D-1 dopamine receptors and activity in the striatum of the rat after magnetic field exposure. Dopamine in the striatum is involved in Parkinson’s disease. Wang et al. (2008) reported that ELF magnetic fields potentiated morphine-induced decrease in D-2 dopamine receptors. The implication of these data is not readily clear. Both D-1 and D-2 dopamine receptors in the brain are involved in depression and drug addiction. There is one study on the cholinergic system. Ravera et al. (2010) reported changes in the enzyme acetylcholinesterase in cell membrane isolated from the cerebellum after magnetic field exposure. Interesting, these researchers also reported ‘frequency window’ effects in their experiment. Window effects, i.e., effects are observed at a certain range(s) of EMF frequency or intensity, were first reported by Ross Adey and Susan Bawin and Carl Blackman in the 1980s. A recently study by Fournier et al. (2012) reported an ‘intensity window’ effect of ELF magnetic field on neurodevelopment in the rat. The cholinergic systems in the brain play a major role in learning and memory functions. There were a

series of studies carried out more than a decade ago showing effects of ELF magnetic field on the cholinergic systems, e.g., Lai and Carino (1999) (60-Hz magnetic field and central cholinergic activity: effects of exposure intensity and duration. Bioelectromagnetics 20:284-289, 1999). Not many studies have been carried out in recent years to further investigate the effects of EMF on this important neurological function.

2. Behavioral effects of ELF EMF have been further substantiated in recent research. These included: changes in locomotor activity (Balassa et al., 2009; Dimitrijevic et al., 2014; Janac et al., 2012; Legros et al., 2012; Raus et al., 2012b; Shin et al., 2007, 2011; Todorovic et al., 2012), learning and memory functions (Che et al., 2007; Corbacio et al., 2011; Cui et al., 2012; Duan et al., 2013; Fournier et al., 2012; Fu et al., 2008; Harakawa et al., 2008; He et al., 2011; Liu et al., 2008b; Sun et al., 2010), anxiety (Balassa et al., 2009; He et al., 2011; Korpinar et al., 2012; Liu et al., 2008a; Salunke et al., 2013); depression-like behavior (Kitaoka et al., 2013; Szemerszky et al., 2011), perception (Ross et al., 2008), cognitive dysfunction (Davanipour et al., 2014), emotional state (Stevens, 2007), sleep onset (Hung et al., 2007), and comb building in hornets (Ishay et al., 2007). Since different behavioral effects have been observed in different exposure conditions, species of animals, and testing paradigms, they provide the strongest evidence that exposure to ELF EMF can affect the nervous system.

3. In some of these observed neurological effects, oxidative changes (free radicals) again seemed to play a role (Akdag et al., 2010, 2013; Akpinar et al., 2013; Cho et al., 2012; Chu et al., 2011; Ciejka et al., 2011; Deng et al., 2013; Coskun et al., 2009; Cui et al., 2012; Cui et al., 2012; Di Loreto et al., 2009; Duan et al., 2013; Falone et al., 2008; Manikonda et al., 2013; Martinez-Samano et al., 2012; Rauš Balind et al., 2014; Selaković et al., 2013; Tassel et al., 2012a, Turkozer et al., 2008). Increase in free radicals causes cellular damages. Most of these effects are changes in enzymes involved in maintenance of oxidative balance in cells. A paper by Falone et al. (2008) reported an interesting finding. The researchers observed that, after magnetic field exposure, the brain of young rats showed an increase in anti-oxidative enzymes and defense against oxidative damage, whereas that of old rat showed a decrease. Thus, aging may make an individual more susceptible to the detrimental effects of ELF EMF. There are other factors that could affect an animal's response to ELF EMF. Janac et al. (2012) reported age-dependent effects of ELF EMF on locomotor activity in the Gerbils. Reyes-Guerrero et al. (2010) found that the fields affected olfactory bulb estrogen receptors in female but not in male rats. Sun et al. (2010) reported that, after in ovo (in the egg) exposure to ELF EMF, chicks showed memory deficit only when they were under stress. Indeed, Lahijani et al. (2011) reported histological changes in the brain of chicks exposed to ELF EMF in ovo.

4. The possible medical applications of ELF EMF should be given more attention. Several studies indicate that ELF EMF could enhance recovery of functions after nervous system damage and have protective effects against development of neurodegenerative diseases. Cuccurazzu et al. (2010) reported an ELF EMF-induced neurogenesis and repair of the nervous system after damage. Kumar et al. (2010) and Das et al. (2012) showed an enhanced restoration of functions after spinal injury in the rat. Kumar et al. (2013) further showed that ELF EMF exposure restored spinal cord injury-induced tonic pain and

changes in neurotransmitter concentrations in the brain of the rat. Maestú et al. (2013) reported improvement in pain sensation in fibromyalgia patients after magnetic field stimulation. A possible beneficial effect on cerebral ischemia has been reported by Rauš Balind et al. (2014). Piacentini et al. (2008) reported a promotion of neural differentiation by ELF EMF. Kim et al. (2013) and Bai et al. (2013) reported stimulation by ELF EMF on neural differentiation of stem cells. Effects on stem cells and hippocampal neurogenesis also have been reported by Podda et al. (2013) and Leone et al. (2014). Protective effects of ELF EMF have been reported by Raus et al (2012a, b) after cerebral ischemia, Tassel et al. (2012a, b) on the development of Huntington's Disease, and Manjhi et al. (2013) on spinal cord injury induced osteoporosis. Furthermore, Cvetkovic et al. (2009) reported alteration of EEG by application of certain frequencies of magnetic fields. This may be useful in the treatment of certain neurological disorders such as sleep and psychiatric disorders. Static magnetic field has been shown by Wang et al. (2010) to act like an anti-Parkinson drug. Static magnetic field also has been shown to have antiangiogenesis property (Wang Z, Yang P, Xu H, Qian A, Hu L, Shang P. Inhibitory effects of a gradient static magnetic field on normal angiogenesis. Bioelectromagnetics. 30(6):446-453, 2009), which can be translated into an anticancer activity. Use of ELF EMF for cancer treatment has been extensively investigated. There is a study showed that pulsed electromagnetic fields turned on adenosine receptors in brain cancer cells that inhibit cancer growth (Vincenzi F, Targa M, Corciulo C, Gessi S, Merighi S, Setti S, Cadossi R, Borea PA, Varani K. The anti-tumor effect of A_3 adenosine receptors is potentiated by pulsed electromagnetic fields in cultured neural cancer cells. PLoS One 7(6):e39317, 2012). Interesting, this effect was not observed when normal brain cells were exposed to magnetic field. The waveform of the fields may play an important role in the effect produced. There are several studies on pulsed (instead of sinusoidal) magnetic fields (Aldinucci et al., 2009; Capone et al., 2009; Cook et al. 2009; Glover et al., 2009) and complex fields (Ross et al., 2008). It has been speculated that intermittent EMF or fields that have a transient nature could be more biologically potent than constant fields. The conditions and parameters of the fields that could produce either detrimental or beneficial effects need further investigation. Furthermore, it is still not clear whether acute (one time) exposure would elicit effects different from chronic/repeated exposure. In the 2007-2012 literature, there are many studies investigated the effects of chronic/repeated exposure. The study by Liu et al. (2008a) indicates that duration of exposure could be an important factor.

5. The majority of the studies used magnetic fields above 0.1 mT (1 gauss; the highest was 8 mT). The intensities are much higher than those in the public environment. Thus, caution should be taken in extrapolating the high-intensity cell and animal studies to environmental human exposure situation. Exposure to magnetic fields of 0.4 µT (0.0004 mT) has been implication in an increased risk of childhood leukemia. And, the recent report by Li et al. (Li DK, Ferber JR, Odouli R, Quesenberry CP Jr. A Prospective Study of In-utero Exposure to Magnetic Fields and the Risk of Childhood Obesity. Sci Rep. 2:540, 2012) on an increased risk of obesity of humans exposed prenatally to magnetic field at 0.25 µT (0.00025 mT). There is also a report of a blood pressure lowering effect in humans with mild-to-moderate hypertension after exposure to magnetic fields at 1 µT (0.001mT) (Nishimura T, Tada H, Guo X, Murayama T, Teramukai S, Okano H, Yamada J, Mohri K, Fukushima M. A 1-µT extremely low-frequency electromagnetic field vs.

sham control for mild-to-moderate hypertension: a double-blind, randomized study. Hypertens Res. 34(3):372-377, 2011.) Apparently, humans are sensitive to magnetic field at level less than 1 µT. There are a study by Ross et al (2008) showing 'perception' alternation in human subjects exposed to magnetic field at 10 nT (0.00001 mT), a study by Fournier et al (2012) on effect of brain development in the rat at 30 nT (0.00003 mT), and a study by Stevens (2007) indicating changes in emotional states in humans exposed to 8-12 Hz magnetic field at 5 µT (0.005 mT). These data do suggest magnetic fields at very low intensities could cause neurological effects in humans. In the 1990s, there was a series of more than 20 studies published by Reuven Sandyk showing that pulsed magnetic fields at pT (1 pT = 0.000000001 mT) levels could have therapeutic effects on Parkinson's disease and multiple sclerosis (see e.g., Sandyk R. Reversal of cognitive impairment in an elderly Parkinsonian patient by transcranial application of picotesla electromagnetic fields. Int J Neurosci. 91(1-2):57-68, 1997, or, search for 'Sandyk R' in the PubMed.) However, Sandyk's findings have never been independently confirmed.

6. In summary, both RF and ELF EMF affect neurological functions and behavior in animals and humans. There is no definite data showing that these effects are detrimental to human health. However, since effects have been observed, it is advisable that one should limit one's exposure to EMF.

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12. In summary, both RF and ELF EMF affect neurological functions and behavior in animals and humans. There is no definite data showing that these effects are detrimental to human health. However, since effects have been observed, it is advisable that one should limit one's exposure to EMF.

APPENDIX A: ABSTRACTS OF STUDIES ON NEUROLOGICAL EFFECTS OF RADIOFREQUENCY RADIATION (RFR) - (2007-2014)

Below is a key to abbreviations used throughout the following list of abstracts for recent papers published since 2007 and serve as my comments to help the reader identify the significance of each paper.

(E)-effect observed; (NE)- no significant observed; HU- human study; AS- animal study; CS-cell study; LI- low intensity/cell tower; CE- chronic/repeated exposure; BE- behavioral effect; DE- developmental effect; CC- cellular effects; CH-chemical changes; ME-morphological effect; PE-physiological effect; EE- electrophysiological effect; OX-oxidative changes; AD- age-dependent effect; SL- effect on sleep; MA- possible medical application; WS- waveform specific effect; IA- interaction with other factors.

(E) Abdel-Rassoul G, El-Fateh OA, Salem MA, Michael A, Farahat F, El-Batanouny M, Salem E. Neurobehavioral effects among inhabitants around mobile phone base stations. Neurotoxicology. 28(2):434-440, 2007. (HU, CE, BE, LI, SL)

S

(E) Aboul Ezz HS, Khadrawy YA, Ahmed NA, Radwan NM, El Bakry MM. The effect of pulsed electromagnetic radiation from mobile phone **on the levels of monoamine neurotransmitters in four different areas of rat brain. Eur Rev Med Pharmacol Sci. 17(13):1782-1788, 2013. (AS, CE, CH)**

BACKGROUND: The use of mobile phones is rapidly increasing all over the world. Few studies deal with the effect of electromagnetic radiation (EMR) on monoamine neurotransmitters in the different brain areas of adult rat. AIM: The aim of the present study was to investigate the effect of EMR on the concentrations of dopamine (DA), norepinephrine (NE) and serotonin (5-HT) in the hippocampus, hypothalamus, midbrain and medulla oblongata of adult rats. MATERIALS AND METHODS: Adult rats were exposed daily to EMR (frequency 1800 MHz, specific absorption rate 0.843 W/kg, power density 0.02 mW/cm2, modulated at 217 Hz) and sacrificed after 1, 2 and 4 months of daily EMR exposure as well as after stopping EMR for 1 month (after 4 months of daily EMR exposure). Monoamines were determined by high performance liquid chromatography coupled with fluorescence detection (HPLC-FD) using their native properties. RESULTS: The exposure to EMR resulted in significant changes in DA, NE and 5-HT in the four selected areas of adult rat brain. CONCLUSIONS: The exposure of adult rats to EMR may cause disturbances in monoamine neurotransmitters and this may underlie many of the adverse effects reported after EMR including memory, learning, and stress.

***(E) Abramson MJ, Benke GP, Dimitriadis C, Inyang IO, Sim MR, Wolfe RS, Croft RJ. Mobile telephone use is associated with changes in cognitive function in young adolescents. Bioelectromagnetics. 30(8):678-686, 2009. (HU, BE) (*Effects observed probably not caused by exposure to RFR.)**

As part of the Mobile Radiofrequency Phone Exposed Users' Study (MoRPhEUS), a cross-sectional epidemiological study examined cognitive function in secondary school students. We recruited 317, 7th grade students (144 boys, 173 girls, median age 13 years) from 20 schools around Melbourne, Australia. Participants completed an exposure questionnaire based on the Interphone study, a computerised cognitive test battery, and the Stroop colour-word test. The principal exposure metric was the total number of reported mobile phone voice calls per week. Linear regression models were fitted to cognitive test response times and accuracies. Age, gender, ethnicity, socio-economic status and handedness were fitted as covariates and standard errors were adjusted for clustering by school. The accuracy of working memory was poorer, reaction time for a simple learning task shorter, associative learning response time shorter and accuracy poorer in children reporting more mobile phone voice calls. There were no significant relationships between exposure and signal detection, movement monitoring or estimation. The completion time for Stroop word naming tasks was longer for those reporting more mobile phone voice calls. The findings were similar for total short message service (SMS, also known as text) messages per week, suggesting these cognitive changes were unlikely due to radiofrequency (RF) exposure. Overall, mobile phone use was associated with faster and less accurate responding to higher level cognitive tasks. These behaviours may have been learned through frequent use of a mobile phone.

(NE) Ahlers MT, Ammermüller J. No influence of acute RF exposure (GSM-900, GSM-1800, and UMTS) on mouse retinal ganglion cell responses under constant temperature conditions. Bioelectromagnetics. 2013 Sep 21. doi: 10.1002/bem.21811. [Epub ahead of print] (CS, CC)

Possible non-thermal effects of radio frequency electromagnetic fields (RF-EMF) on retinal ganglion cells were studied in vitro under conditions of constant temperature. Isolated mouse retinae were exposed to GSM-900, GSM-1800, and universal mobile telecommunication system (UMTS) RF-EMF applying specific absorption rates (SAR) of 0 (sham), 0.02, 0.2, 2, and 20 W/kg. Temperature was kept constant within ±0.5 to 1 °C for GSM-900 and ±0.5 °C for GSM-1800 and UMTS. Responses of retinal ganglion cells to light stimuli of three intensities (0.5, 16, and 445 lx) were recorded before, during, and up to 35 min after exposure. Experiments were performed under double-blind conditions. Changes in light responses during and after exposure were determined for each condition (RF-EMF; SAR value; light intensity) with respect to the responses before exposure, respectively. Changes were calculated using the Euclidian distance of the n-dimensional response vectors, respectively. Some changes already occurred during sham (0 W/kg) exposure, reflecting the intrinsic variability in retinal ganglion cell responses. Comparison of the distance values from sham exposure with those from actual exposure yielded no significant differences. In addition, linear regression analysis of the distance values versus SAR values yielded no consistent dependence of light response changes. From these results we conclude that RF-EMF exposure at three mobile phone frequencies (GSM-900, GSM-1800, UMTS) and SARs up to 20 W/kg has no acute effects on retinal ganglion cell responses under constant temperature conditions.

(NE) Aït-Aïssa S, de Gannes FP, Taxile M, Billaudel B, Hurtier A, Haro E, Ruffié G, Athané A, Veyret B, Lagroye I. In Situ Expression of Heat-Shock Proteins and 3-Nitrotyrosine in Brains of Young Rats Exposed to a WiFi Signal In Utero and In Early Life. Radiat Res. 2013 May 10. [Epub ahead of print] (AS, CE, CH, DE, OX)

The bioeffects of exposure to Wireless High-Fidelity (WiFi) signals on the developing nervous systems of young rodents was investigated by assessing the in vivo and in situ expression levels of three stress markers: 3-Nitrotyrosine (3-NT), an oxidative stress marker and two heat-shock proteins (Hsp25 and Hsp70). These biomarkers were measured in the brains of young rats exposed to a 2450 MHz WiFi signal by immunohistochemistry. Pregnant rats were first exposed or sham exposed to WiFi from day 6 to day 21 of gestation. In addition three newborns per litter were further exposed up to 5 weeks old. Daily 2-h exposures were performed blind in a reverberation chamber and whole-body specific absorption rate levels were 0, 0.08, 0.4 and 4 W/kg. 3-NT and stress protein expression was assayed in different areas of the hippocampus and cortex. No significant difference was observed among exposed and sham-exposed groups. These results suggest that repeated exposure to WiFi during gestation and early life has no deleterious effects on the brains of young rats.

(E) Aldad TS, Gan G, Gao XB, Taylor HS. Fetal radiofrequency radiation exposure from 800-1900 MHz-rated cellular telephones affects neurodevelopment and behavior in mice. Sci Rep. 2:312, 2012. (AS, CS, DE, BE, CE, CC)

Neurobehavioral disorders are increasingly prevalent in children, however their etiology is not well understood. An association between prenatal cellular telephone use and hyperactivity in children has been postulated, yet the direct effects of radiofrequency radiation exposure on neurodevelopment remain unknown. Here we used a mouse model to demonstrate that in-utero radiofrequency exposure from cellular telephones does affect adult behavior. Mice exposed in-utero were hyperactive and had impaired memory as determined using the object recognition, light/dark box and step-down assays. Whole cell patch clamp recordings of miniature excitatory postsynaptic currents (mEPSCs) revealed that these behavioral changes were due to altered neuronal developmental programming. Exposed mice had dose-responsive impaired glutamatergic synaptic transmission onto layer V pyramidal neurons of the prefrontal cortex. We present the first experimental evidence of neuropathology due to in-utero cellular telephone radiation. Further experiments are needed in humans or non-human primates to determine the risk of exposure during pregnancy.

(E) Ammari M, Brillaud E, Gamez C, Lecomte A, Sakly M, Abdelmelek H, de Seze R. Effect of a chronic GSM 900 MHz exposure on glia in the rat brain. Biomed Pharmacother. 62(4):273-281, 2008a. (AS, CE, CC)

Extension of the mobile phone technology raises concern about the health effects of 900 MHz microwaves on the central nervous system (CNS). In this study we measured GFAP expression using immunocytochemistry method, to evaluate glial evolution 10 days after a chronic exposure (5 days a week for 24 weeks) to GSM signal for 45 min/day at a brain-averaged specific absorption rate (SAR)=1.5 W/kg and for 15 min/day at a SAR=6 W/kg in the following rat brain areas: prefrontal cortex (PfCx), caudate putamen (Cpu), lateral globus pallidus of striatum (LGP), dentate gyrus of hippocampus (DG) and cerebellum cortex (CCx). In comparison to sham or cage control animals, rats exposed to chronic GSM signal at 6 W/kg have increased GFAP stained surface areas in the brain ($p<0.05$). But the chronic exposure to GSM at 1.5 W/kg did not increase GFAP expression. Our results indicated that chronic exposure to GSM 900 MHz microwaves (SAR=6 W/kg) may induce persistent astroglia activation in the rat brain (sign of a potential gliosis).

(E) Ammari M, Lecomte A, Sakly M, Abdelmelek H, de-Seze R. Exposure to GSM 900 MHz electromagnetic fields affects cerebral cytochrome c oxidase activity. Toxicology. 250(1):70-74, 2008b. (AS, CE, CH)

The world-wide and rapidly growing use of mobile phones has raised serious concerns about the biological and health-related effects of radio frequency (RF) radiation, particularly concerns about the effects of RFs upon the nervous system. The goal of this study was conducted to measure cytochrome oxidase (CO) levels using histochemical methods in order to evaluate regional brain metabolic activity in rat brain after exposure to a GSM 900 MHz signal for 45 min/day at a brain-averaged specific absorption rate (SAR) of 1.5 W/Kg or for 15 min/day at a SAR of 6 W/Kg over seven days. Compared to the sham and control cage groups, rats exposed to a GSM signal at 6 W/Kg showed decreased CO activity in some areas of the prefrontal and frontal cortex (infralimbic cortex, prelimbic cortex, primary motor cortex, secondary motor cortex, anterior cingulate cortex areas 1 and 2 (Cg1 and Cg2)), the septum (dorsal and ventral parts of the lateral septal nucleus), the hippocampus (dorsal field CA1, CA2 and CA3 of the hippocampus and dental gyrus) and the posterior cortex (retrosplenial agranular cortex, primary and secondary visual cortex, perirhinal cortex and lateral entorhinal cortex). However, the exposure to GSM at 1.5 W/Kg did not affect brain activity. Our results indicate that 6 W/Kg GSM 900 MHz microwaves may affect brain metabolism and neuronal activity in rats.

(NE) Ammari M, Jacquet A, Lecomte A, Sakly M, Abdelmelek H, de Seze R. Effect of head-only sub-chronic and chronic exposure to 900-MHz GSM electromagnetic fields on spatial memory in rats. Brain Inj. 22(13-14):1021-1029, 2008c. (AS, CE, BE)

PRIMARY OBJECTIVE: This study was carried out to investigate the behavioural effects of sub-chronic and chronic head-only exposure to 900 MHz GSM (Global System for Mobile communications) in male rats. **METHODS:** Rats were exposed for 45 minutes per day, at a brain-averaged specific absorption rate (SAR) = 1.5 W Kg(-1) or 15 minutes per day at a SAR = 6 W Kg(-1), during 8 or 24 weeks. Then, their spatial memory was tested using the radial-arm maze. In the first phase (10 days), rats were trained to visit the eight arms of the maze without returning to an arm already visited. In the second phase (8 days), a 45-minute intra-trial delay was introduced after four visited arms. **RESULTS:** Performance of exposed rats (1.5 or 6 W Kg(-1)) was compared with that of sham, negative control and positive control rats. Scopolamine treatment in the positive control rats induced deficit in spatial memory task in the second phase of the test. However, spatial memory task was unaffected in exposed rats. **CONCLUSION:** Sub-chronic and chronic head-only exposure of rats to GSM 900 MHz signal (45-minutes, SAR = 1.5 or 15-minutes, SAR = 6 W Kg(-1)) did not induce spatial memory deficit in the radial-arm maze.

(E) Ammari M, Gamez C, Lecomte A, Sakly M, Abdelmelek H, De Seze R. GFAP expression in the rat brain following sub-chronic exposure to a 900 MHz electromagnetic field signal. Int J Radiat Biol. 86(5):367-375, 2010. (AS, CE, CC)

PURPOSE: The rapid development and expansion of mobile communications contributes to the general debate on the effects of electromagnetic fields emitted by mobile phones on the nervous system. This study aims at measuring the glial fibrillary acidic protein (GFAP) expression in 48 rat brains to evaluate reactive astrocytosis, three and 10 days after long-term head-only sub-chronic exposure to a 900 MHz electromagnetic field (EMF) signal, in male rats. **METHODS:** Sprague-Dawley rats were exposed for 45 min/day at a brain-averaged specific absorption rate (SAR) = 1.5 W/kg or 15 min/day at a SAR = 6 W/kg for five days per week during an eight-week period. GFAP expression was measured by the immunocytochemistry method in the following rat brain areas: Prefrontal cortex, cerebellar cortex, dentate gyrus of the hippocampus, lateral globus pallidus of the striatum, and the caudate putamen. **RESULTS:** Compared to the sham-treated rats, those exposed to the sub-chronic GSM (Global System for mobile communications) signal at 1.5 or 6 W/kg showed an increase in GFAP levels in the different brain areas, three and ten days after treatment. **CONCLUSION:** Our results show that sub-chronic exposures to a 900 MHz EMF signal for two months could adversely affect rat brain (sign of a potential gliosis).

(E) Arendash GW, Sanchez-Ramos J, Mori T, Mamcarz M, Lin X, Runfeldt M, Wang L, Zhang G, Sava V, Tan J, Cao C. Electromagnetic field treatment protects against and reverses cognitive impairment in Alzheimer's disease mice. J Alzheimers Dis. 19(1):191-210, 2010. (AS, CE, CH, BE, MA)

Despite numerous studies, there is no definitive evidence that high-frequency electromagnetic field (EMF) exposure is a risk to human health. To the contrary, this report presents the first evidence that long-term EMF exposure directly associated with cell phone use (918 MHz; 0.25 w/kg) provides cognitive benefits. Both cognitive-protective and cognitive-enhancing effects of EMF exposure were discovered for both normal mice and transgenic mice destined to develop Alzheimer's-like cognitive impairment. The cognitive interference task utilized in this study was designed from, and measure-for-measure analogous to, a human cognitive interference task. In Alzheimer's disease mice, long-term EMF exposure reduced brain amyloid-beta (Abeta) deposition through Abeta anti-aggregation actions and increased brain temperature during exposure periods. Several inter-related mechanisms of EMF action are proposed, including increased Abeta clearance from the brains of Alzheimer's disease mice, increased neuronal activity, and increased cerebral blood flow. Although caution should be taken in extrapolating these mouse studies to humans, we conclude that EMF exposure may represent a non-invasive, non-pharmacologic therapeutic against Alzheimer's disease and an effective memory-enhancing approach in general.

(E) Arendash GW, Mori T, Dorsey M, Gonzalez R, Tajiri N, Borlongan C. Electromagnetic treatment to old Alzheimer's mice reverses β-amyloid deposition, modifies cerebral blood flow, and provides selected cognitive benefit. PLoS One. 7(4):e35751, 2012. (AS, CE, CH, BE, MA)

Few studies have investigated physiologic and cognitive effects of "long-term" electromagnetic field (EMF) exposure in humans or animals. Our recent studies have provided initial insight into the long-term impact of adulthood EMF exposure (GSM, pulsed/modulated, 918 MHz, 0.25-1.05 W/kg) by showing 6+ months of daily EMF treatment protects against or reverses cognitive impairment in Alzheimer's transgenic (Tg) mice, while even having cognitive benefit to normal mice. Mechanistically, EMF-induced cognitive benefits involve suppression of brain β-amyloid (Aβ) aggregation/deposition in Tg mice and brain mitochondrial enhancement in both Tg and normal mice. The present study extends this work by showing that daily EMF treatment given to very old (21-27 month) Tg mice over a 2-month period reverses their very advanced brain Aβ aggregation/deposition. These very old Tg mice and their normal littermates together showed an increase in general memory function in the Y-maze task, although not in more complex tasks. Measurement of both body and brain temperature at intervals during the 2-month EMF treatment, as well as in a separate group of Tg mice during a 12-day treatment period, revealed no appreciable increases in brain temperature (and no/slight increases in body temperature) during EMF "ON" periods. Thus, the neuropathologic/cognitive benefits of EMF treatment occur without brain hyperthermia. Finally, regional cerebral blood flow in cerebral cortex was determined to be reduced in both Tg and normal mice after 2 months of EMF treatment, most probably through cerebrovascular constriction induced by freed/disaggregated Aβ (Tg mice) and slight body hyperthermia during "ON" periods. These results demonstrate that long-term EMF treatment can provide general cognitive benefit to very old Alzheimer's Tg mice and normal mice, as well as reversal of advanced Aβ neuropathology in Tg mice without brain heating. Results further underscore the potential for EMF treatment against AD.

***(E) Arns M, Van Luijtelaar G, Sumich A, Hamilton R, Gordon E. Electroencephalographic, personality, and executive function measures associated with frequent mobile phone use. Int J Neurosci. 117(9):1341-1360, 2007. (HU, BE) (*Effects observed probably not caused by exposure to RFR.)**

The present study employs standardized data acquired from the Brain Resource International Database to study the relationship between mobile phone usage, personality, and brain function (n = 300). Based on the frequency and duration of mobile phone usage, three groups were formed. The findings suggest a subtle slowing of brain activity related to mobile phone use that is not explained by differences in personality. These changes are still within normal physiological ranges. Better executive function in mobile phone users may reflect more focused attention, possibly associated with a cognitive training effect (i.e., frequently making phone calls in distracting places), rather than a direct effect of mobile phone use on cognition.

(E) Bak M, Dudarewicz A, Zmyślony M, Sliwinska-Kowalska M. Effects of GSM signals during exposure to event related potentials (ERPs). Int J Occup Med Environ Health. 23(2):191-199, 2010. (HU, EE)

OBJECTIVES: The primary aim of this work was to assess the effect of electromagnetic field (EMF) from the GSM mobile phone system on human brain function. The assessment was based on the assay of event related potentials (ERPs). **MATERIAL AND METHODS:** The study group consisted of 15 volunteers, including 7 men and 8 women. The test protocol comprised determination of P300 wave in each volunteer during exposure to the EMF. To eliminate possible effects of the applied test procedure on the final result, the test was repeated without EMF exposure. P300 latency, amplitude, and latency of the N1, N2, P2 waves were analysed. **RESULTS:** The statistical analysis revealed an effect of EMF on P300 amplitude. In the experiment with EMF exposure, lower P300 amplitudes were observed only at the time in which the volunteers were exposed to EMF; when the exposure was discontinued, the values of the amplitude were the same as those observed before EMF application. No such change was observed when the experiment was repeated with sham exposure, which may be considered as an indirect proof that lower P300 amplitude values were due to EMF exposure. No statistically significant changes were noted in the latencies of the N1, N2, P2 waves that precede the P300 wave, nor in the latency of the P300 itself. **CONCLUSIONS:** The results suggest that exposure to GSM EMF exerts some effects on CNS, including effects on long latency ERPs.

(E) Barcal J, Vozeh F. Effect of whole-body exposure to high-frequency electromagnetic field on the brain cortical and hippocampal activity in mouse experimental model. NeuroQuantology 5:292-302, 2007. (AS, EE)

Evaluation of the direct registration of brain cortical and hippocampal activity during a high-frequency electromagnetic field (HF-EMF) exposure was performed. Experimental procedures were done under general anesthesia (urethane, 20%, 2g/kg i.p.) in Lurcher mutant mice, wild type (healthy littermates) were used as controls. Animals were exposed to the HF-EMF with frequency corresponding to cellular phones (900 MHz). We used of gel electrodes (silicon tubes or glass microcapillary filled with agar) where the connection with classical electrodes was located out of HF-EMF space. ECoG evaluation showed a distinct shift to lower frequency components but clear effect has been observed only in wild type (healthy) mice whereas in Lurcher mutant mice only gentle differences between frequency spectra were found. Measurement of hippocampal rhythmicity showed gentle changes with increase of higher frequencies (i.e. opposite effect than in cortex) and changes in theta oscillations registered from a dentate gyrus and CA1 area in both types of animals (healthy and mutant). These findings support an idea about possible influencing the central nervous system by HF-EMF exposure and support also some recent results about possible health risks resulting from cellular phones use.

(E) Bas O, Odaci E, Kaplan S, Acer N, Ucok K, Colakoglu S. 900 MHz electromagnetic field exposure affects qualitative and quantitative features of hippocampal pyramidal cells in the adult female rat. Brain Res. 1265:178-185, 2009. (AS, CE, ME)

The effects of electromagnetic fields (EMFs) emitted by mobile phones on humans hold special interest due to their use in close proximity to the brain. The current study investigated the number of pyramidal cells in the cornu ammonis (CA) of the 16-week-old female rat hippocampus following postnatal exposure to a 900 megahertz (MHz) EMF. In this study were three groups of 6 rats: control (Cont), sham exposed (Sham), and EMF exposed (EMF). EMF group rats were exposed to 900 MHz EMF (1 h/day for 28 days) in an exposure tube. Sham group was placed in the exposure tube but not exposed to EMF (1 h/day for 28 days). Cont group was not placed into the exposure tube nor were they exposed to EMF during the study period. In EMF group rats, the specific energy absorption rate (SAR) varied between 0.016 (whole body) and 2 W/kg (locally in the head). All of the rats were sacrificed at the end of the experiment and the number of pyramidal cells in the CA was estimated using the optical fractionator technique. Histopathological evaluations were made on sections of the CA region of the hippocampus. Results showed that postnatal EMF exposure caused a significant decrease of the pyramidal cell number in the CA of the EMF group ($P<0.05$). Additionally, cell loss can be seen in the CA region of EMF group even at qualitative observation. These results may encourage researchers to evaluate the chronic effects of 900 MHz EMF on teenagers' brains.

(E) Baş O, Sönmez OF, Aslan A, İkinci A, Hancı H, Yıldırım M, Kaya H, Akça M, Odacı E. Pyramidal Cell Loss in the Cornu Ammonis of 32-day-old Female Rats Following Exposure to a 900 Megahertz Electromagnetic Field During Prenatal Days 13–21. NeuroQuantology 11:591-599, 2013. (AS, CE, ME, DE)

The number of studies reporting that the electromagnetic field (EMF) emitted by mobile phones affects human health is increasing by the day. In previous studies we reported that a 900 megahertz (MHz) EMF applied throughout the prenatal period reduced the number of pyramidal cells in the cornu ammonis of rat pups in the postnatal period. In this study we investigated the effect of a 900 MHz EMF applied on days 13-21 of the prenatal period on the number of pyramidal cells in the cornu ammonis of rat pups in the postnatal period. For that purpose, pregnant rats were divided into experimental and control groups. Experimental group pregnant rats were exposed to the effect of a 900 MHz EMF on days 13-21 of pregnancy. No procedure was applied to the control group. Newborn female rat pups were added to the study, and no procedure was performed on these after birth. Five newborn female rats were obtained from the experimental group and six from the control group. All female rat pups were decapitated on the postnatal 32nd day, and histological procedures were performed on the brain tissues. Sections were stained with Cresyl fast violet. The optical dissector technique was used to estimate the total number of pyramidal cells in the cornu ammonis. Sections of cornu ammonis were subjected to histopathological evaluations. Our results showed that exposure to 900 MHz EMF during prenatal days 13-21 led to a significant decrease in the number of pyramidal cells in the cornu ammonis of the experimental group female rat pups ($P<0.05$). Histopathological examination revealed picnotic cells in the cornu ammonis in experimental female rat pups. The pyramidal cell loss in the cornu ammonis may therefore be attributed to exposure to 900 MHz EMF in days 13-21 of the prenatal period.

(E) Bodera P, Stankiewicz W, Antkowiak B, Paluch M, Kieliszek J, Sobiech J, Zdanowski R, Wojdas A, Siwicki AK, Skopińska-Rózewska E. Suppressive effect of electromagnetic field on analgesic activity of tramadol in rats. Pol J Vet Sci. 15(1):95-100, 2012. (AS, PE, IA)

The electromagnetic fields (EMFs) have been shown to alter animal and human behavior, such as directional orientation, learning, pain perception (nociception or analgesia) and anxiety-related behaviors. The aim of this study was to evaluate the influence of electromagnetic fields of high-frequency microwaves on pain perception and anti-nociceptive activity of tramadol (TRAM) - analgetic effective in the treatment of moderate to severe acute and chronic pain states. Electromagnetic fields exposures of a)1500 MHz frequency and b) modulated, 1800 MHz (which is identical to that generated by mobile phones) were applied. Paw withdrawal latency (PWL) to thermal stimulus was measured in vehicle or tramadol (TRAM) treated animals before and after 30, 60 and 90 minutes from injections. The differences in the level of pain (PWL) between control group and rats exposed to EMF alone in three measurements, were not observed. Tramadol alone significantly increased PWLs to thermal stimulus in comparison to vehicle results at 30 ($p < 0.001$) and 60 minutes ($p < 0.05$) after drug injection. EMF exposure of both frequencies transiently suppressed analgesic effect of tramadol, significantly reducing paw withdrawal latency in animals treated with this drug at 30 minutes from the drug injection.

(E) Bouji M, Lecomte A, Hode Y, de Seze R, Villégier AS. Effects of 900 MHz radiofrequency on corticosterone, emotional memory and neuroinflammation in middle-aged rats. Exp Gerontol. 47(6):444-451, 2012. (AS, CC, BE, AD)

The widespread use of mobile phones raises the question of the effects of electromagnetic fields (EMF, 900 MHz) on the brain. Previous studies reported increased levels of the glial fibrillary acidic protein (GFAP) in the rat's brain after a single exposure to 900 MHz global system for mobile (GSM) signal, suggesting a potential inflammatory process. While this result was obtained in adult rats, no data is currently available in older animals. Since the transition from middle-age to senescence is highly dependent on environment and lifestyle, we studied the reactivity of middle-aged brains to EMF exposure. We assessed the effects of a single 15 min GSM exposure (900 MHz; specific absorption rate (SAR)=6 W/kg) on GFAP expression in young adults (6 week-old) and middle-aged rats (12 month-old). Brain interleukin (IL)-1β and IL-6, plasmatic levels of corticosterone (CORT), and emotional memory were also assessed. Our data indicated that, in contrast to previously published work, acute GSM exposure did not induce astrocyte activation. Our results showed an IL-1β increase in the olfactory bulb and enhanced contextual emotional memory in GSM-exposed middle-aged rats, and increased plasmatic levels of CORT in GSM-exposed young adults. Altogether, our data showed an age dependency of reactivity to GSM exposure in neuro-immunity, stress and behavioral parameters. Reproducing these effects and studying their mechanisms may allow a better understanding of mobile phone EMF effects on neurobiological parameters.

(E) Brillaud E, Piotrowski A, de Seze R. Effect of an acute 900 MHz GSM exposure on glia in the rat brain: a time-dependent study. Toxicology. 238(1):23-33, 2007. (AS, CC)

Because of the increasing use of mobile phones, the possible risks of radio frequency electromagnetic fields adverse effects on the human brain has to be evaluated. In this work we measured GFAP expression, to evaluate glial evolution 2, 3, 6 and 10 days after a single GSM exposure (15min, brain averaged SAR=6W/kg, 900 MHz signal) in the rat brain. A statistically significant increase of GFAP stained surface area was observed 2 days after exposure in the frontal cortex and the caudate putamen. A smaller statistically significant increase was noted 3 days after exposure in the same areas and in the cerebellum cortex. Our results confirm the Mausset-Bonnefont et al. study [Mausset-Bonnefont, A.L., Hirbec, H., Bonnefont, X., Privat, A., Vignon, J., de Seze, R., 2004. Acute exposure to GSM 900MHz electromagnetic fields induces glial reactivity and biochemical modifications in the rat brain. Neurobiol. Dis. 17, 445-454], showing the existence of glial reactivity after a 15min GSM acute exposure at a brain averaged SAR of 6W/kg. We conclude to a temporary effect, probably due to a hypertrophy of glial cells, with a temporal and a spatial modulation of the effect. Whether this effect could be harmful remains to be studied.

(E) Calabrò E, Condello S, Currò M, Ferlazzo N, Caccamo D, Magazù S, Ientile R. Modulation of heat shock protein response in SH-SY5Y by mobile phone microwaves. World J Biol Chem. 3(2):34-40, 2012. (CS, CH)

AIM: To investigate putative biological damage caused by GSM mobile phone frequencies by assessing electromagnetic fields during mobile phone working. METHODS: Neuron-like cells, obtained by retinoic-acid-induced differentiation of human neuroblastoma SH-SY5Y cells, were exposed for 2 h and 4 h to microwaves at 1800 MHz frequency bands. RESULTS: Cell stress response was evaluated by MTT assay as well as changes in the heat shock protein expression (Hsp20, Hsp27 and Hsp70) and caspase-3 activity levels, as biomarkers of apoptotic pathway. Under our experimental conditions, neither cell viability nor Hsp27 expression nor caspase-3 activity was significantly changed. Interestingly, a significant decrease in Hsp20 expression was observed at both times of exposure, whereas Hsp70 levels were significantly increased only after 4 h exposure. CONCLUSION: The modulation of the expression of Hsps in neuronal cells can be an early response to radiofrequency microwaves.

(E) Cammaerts MC, De Doncker P, Patris X, Bellens F, Rachidi Z, Cammaerts D. GSM 900 MHz radiation inhibits ants' association between food sites and encountered cues. Electromagn Biol Med. 31(2):151-165, 2012. (AS, BE)

The kinetics of the acquisition and loss of the use of olfactory and visual cues were previously obtained in six experimental colonies of the ant Myrmica sabuleti meinert 1861, under normal conditions. In the present work, the same experiments were conducted on six other naive identical colonies of M. sabuleti, under electromagnetic radiation similar to those surrounding GSM and communication masts. In this situation, no association between food and either olfactory or visual cues occurred. After a recovery period, the ants were able to make such an association but never reached the expected score. Such ants having acquired a weaker olfactory or visual score and still undergoing olfactory or visual training were again submitted to electromagnetic waves. Not only did they lose all that they had memorized, but also they lost it in a few hours instead of in a few days (as under normal conditions when no longer trained). They kept no visual memory at all (instead of keeping 10% of it as they normally do). The impact of GSM 900 MHz radiation was greater on the visual memory than on the olfactory one. These communication waves may have such a disastrous impact on a wide range of insects using olfactory and/or visual memory, i.e., on bees.

(E) Cammaerts MC, Rachidi Z, Bellens F, De Doncker P. Food collection and response to pheromones in an ant species exposed to electromagnetic radiation. Electromagn Biol Med. 2013 Jan 15. [Epub ahead of print] (AS, BE)

We used the ant species Myrmica sabuleti as a model to study the impact of electromagnetic waves on social insects' response to their pheromones and their food collection. We quantified M. sabuleti workers' response to their trail, area marking and alarm pheromone under normal conditions. Then, we quantified the same responses while under the influence of electromagnetic waves. Under such an influence, ants followed trails for only short distances, no longer arrived at marked areas and no longer orientated themselves to a source of alarm pheromone. Also when exposed to electromagnetic waves, ants became unable to return to their nest and recruit congeners; therefore, the number of ants collecting food increases only slightly and slowly. After 180 h of exposure, their colonies deteriorated. Electromagnetic radiation obviously affects social insects' behavior and physiology.

(E) Carballo-Quintás M, Martínez-Silva I, Cadarso-Suárez C, Alvarez-Figueiras M, Ares-Pena FJ, López-Martín E. A study of neurotoxic biomarkers, c-fos and GFAP after acute exposure to GSM radiation at 900 MHz in the picrotoxin model of rat brains. Neurotoxicology. 32(4):478-494, 2011. (AS, CH)

The acute effects of microwave exposure from the Global System for Mobile Communication (GSM) were studied in rats, using 900 MHz radiation at an intensity similar to mobile phone emissions. Acute subconvulsive doses of picrotoxin were then administered to the rats and an experimental model of seizure-proneness was created from the data. Seventy-two adult male Sprague-Dawley rats underwent immunochemical testing of relevant anatomical areas to measure induction of the c-fos neuronal marker after 90min and 24h, and of the glial fibrillary acidic protein (GFAP) 72h after acute exposure to a 900MHz electromagnetic field (EMF). The experimental set-up facilitated measurement of absorbed power, from which the average specific absorption rate was calculated using the finite-difference time-domain (FDTD) 2h after exposure to EMF radiation at 1.45W/kg in picrotoxin-treated rats and 1.38W/kg in untreated rats. Ninety minutes after radiation high levels of c-fos expression were recorded in the neocortex and paleocortex along with low hippocampus activation in picrotoxin treated animals. Most brain areas, except the limbic cortical region, showed important increases in neuronal activation 24h after picrotoxin and radiation. Three days after picrotoxin treatment, radiation effects were still apparent in the neocortex, dentate gyrus and CA3, but a significant decrease in activity was noted in the piriform and entorhinal cortex. During this time, glial reactivity increased with every seizure in irradiated, picrotoxin-treated brain regions. Our results reveal that c-fos and glial markers were triggered by the combined stress of non-thermal irradiation and the toxic effect of picrotoxin on cerebral tissues.

(E) Cetin H, Nazıroğlu M, Celik O, Yüksel M, Pastacı N, Ozkaya MO. Liver antioxidant stores protect the brain from electromagnetic radiation (900 and 1800 MHz)-induced oxidative stress in rats during pregnancy and the development of offspring. J Matern Fetal Neonatal Med. 2014 Mar 3. [Epub ahead of print] (AS, CE, CH, OX, DE)

Objectives: The present study determined the effects of mobile phone (900 and 1800 MHz)-induced electromagnetic radiation (EMR) exposure on oxidative stress in the brain and liver as well as the element levels in growing rats from pregnancy to 6 weeks of age. Methods: Thirty-two rats and their offspring were equally divided into 3 different groups: the control, 900 MHz, and 1800 MHz groups. The 900 MHz and 1800 MHz groups were exposed to EMR for 60 min/day during pregnancy and neonatal development. At the 4th, 5th, and 6th weeks of the experiment, brain samples were obtained. Results: Brain and liver glutathione peroxidase (GSH-Px) activities, as well as liver vitamin A and β-carotene concentrations decreased in the EMR groups, although brain iron, vitamin A, and β-carotene concentrations increased in the EMR groups. In the 6th week, selenium concentrations in the brain decreased in the EMR groups. There were no statistically significant differences in glutathione, vitamin E, chromium, copper, magnesium, manganese, and zinc concentrations between the 3 groups. Conclusion: EMR-induced oxidative stress in the brain and liver was reduced during the development of offspring. Mobile phone-induced EMR could be considered as a cause of oxidative brain and liver injury in growing rats.

(NE) Cinel C, Boldini A, Russo R, Fox E. Effects of mobile phone electromagnetic fields on an auditory order threshold task. Bioelectromagnetics. 28(6):493-496, 2007. (HU, BE)

The effect of acute exposure to radio frequency electromagnetic fields (RF EMF) generated by mobile phones on an auditory threshold task was investigated. 168 participants performed the task while exposed to RF EMF in one testing session (either global system for mobile communication (GSM) or unmodulated signals) while in a separate session participants were exposed to sham signals. Lateralization effects were tested by exposing participants either on the left side or on the right side of the head. No significant effect of exposure to RF EMF was detected, suggesting that acute exposure to RF EMFs does not affect performance in the order threshold task.

(NE) Cinel C, Russo R, Boldini A, Fox E. Exposure to mobile phone electromagnetic fields and subjective symptoms: a double-blind study. Psychosom Med. 70(3):345-348, 2008. (HU, BE)

OBJECTIVES: The objective of this study was to examine whether acute exposure to radio frequency electromagnetic fields (REFs) emitted by mobile phone may affect subjective symptoms. METHODS: Three large groups of volunteers (total 496) were exposed to REFs emitted by mobile phones in one session and sham signals in a different session. REF and sham exposure sessions were counterbalanced and double blinded. Participants were exposed to either Global System for Mobile Communication (GSM) or unmodulated signals, and the mobile phone was positioned either on the left or on the right side of the head. Before and after REF and sham exposure participants completed a questionnaire to rate five symptoms. Any changes in the severity of the symptoms after REF exposure were compared with changes after sham exposure. RESULTS: For one group of participants (N = 160), it was found that dizziness was affected by GSM exposure, but this was not consistently found with the other two groups of participants. No other significant effects were found. CONCLUSIONS: We did not find consistent evidence suggesting that exposure to mobile phone REFs affect subjective symptoms. Even though we acknowledge that more research is needed, we believe that our results give an important contribution to the research on mobile phone use and subjective symptoms.

(E) Croft RJ, Hamblin DL, Spong J, Wood AW, McKenzie RJ, Stough C. The effect of mobile phone electromagnetic fields on the alpha rhythm of human electroencephalogram. Bioelectromagnetics. 29(1):1-10, 2008. (HU, EE)

Mobile phones (MP) emit low-level electromagnetic fields that have been reported to affect neural function in humans; however, demonstrations of such effects have not been conclusive. The purpose of the present study was to test one of the strongest findings in the literature; that of increased "alpha" power in response to MP-type radiation. Healthy participants (N = 120) were tested using a double-blind counterbalanced crossover design, with each receiving a 30-min Active and a 30-min Sham Exposure 1 week apart, while electroencephalogram (EEG) data were recorded. Resting alpha power (8-12 Hz) was then derived as a function of time, for periods both during and following exposure. Non-parametric analyses were employed as data could not be normalized. Previous reports of an overall alpha power enhancement during the MP exposure were confirmed (relative to Sham), with this effect larger at ipsilateral than contralateral sites over posterior regions. No overall change to alpha power was observed following exposure cessation; however, there was less alpha power contralateral to the exposure source during this period (relative to ipsilateral). Employing a strong methodology, the current findings support previous research that has reported an effect of MP exposure on EEG alpha power.

(E) Croft RJ, Leung S, McKenzie RJ, Loughran SP, Iskra S, Hamblin DL, Cooper NR. Effects of 2G and 3G mobile phones on human alpha rhythms: Resting EEG in adolescents, young adults, and the elderly. Bioelectromagnetics. 31(6):434-444, 2010. (HU, EE, AD, WS)

The present study was conducted to determine whether adolescents and/or the elderly are more sensitive to mobile phone (MP)-related bioeffects than young adults, and to determine this for both 2nd generation (2G) GSM, and 3rd generation (3G) W-CDMA exposures. To test this, resting alpha activity (8-12 Hz band of the electroencephalogram) was assessed because numerous studies have now reported it to be enhanced by MP exposure. Forty-one 13-15 year olds, forty-two 19-40 year olds, and twenty 55-70 year olds were tested using a double-blind crossover design, where each participant received Sham, 2G and 3G exposures, separated by at least 4 days. Alpha activity, during exposure relative to baseline, was recorded and compared between conditions. Consistent with previous research, the young adults' alpha was greater in the 2G compared to Sham condition, however, no effect was seen in the adolescent or the elderly groups, and no effect of 3G exposures was found in any group. The results provide further support for an effect of 2G exposures on resting alpha activity in young adults, but fail to support a similar enhancement in adolescents or the elderly, or in any age group as a function of 3G exposure.

(NE) Curcio G, Valentini E, Moroni F, Ferrara M, De Gennaro L, Bertini M. Psychomotor performance is not influenced by brief repeated exposures to mobile phones. Bioelectromagnetics. 29(3):237-241, 2008. (HU, BE)

The present study investigated the presence of a cumulative effect of brief and repeated exposures to a GSM mobile phone (902.40 MHz, 217 Hz modulated; peak power of 2 W; average power of 0.25 W; SAR = 0.5 W/kg) on psychomotor functions. To this end, after each of 3 15-min exposures, both an acoustic simple reaction time task (SRTT) and a sequential finger tapping task (SFTT) were administered to 24 subjects. The present study was unable to detect the cumulative effects of brief and repeated EMF exposure on human psychomotor performance, although there was a non-statistical trend to shorter reaction times. In summary, these data show an absence of effects with these particular exposure conditions; however, possible cognitive effects induced by different signal characteristics cannot be excluded.

(E) Curcio G, Ferrara M, Limongi T, Tempesta D, Di Sante G, De Gennaro L, Quaresima V, Ferrari M. Acute mobile phones exposure affects frontal cortex hemodynamics as evidenced by functional near-infrared spectroscopy. J Cereb Blood Flow Metab. 29(5):903-910, 2009. (HU, PE)

This study aimed to evaluate by functional near-infrared spectroscopy (fNIRS), the effects induced by an acute exposure (40 mins) to a GSM (Global System for Mobile Communications) signal emitted by a mobile phone (MP) on the oxygenation of the frontal cortex. Eleven healthy volunteers underwent two sessions (Real and Sham exposure) after a crossover, randomized, double-blind paradigm. The whole procedure lasted 60 mins: 10-mins baseline (Bsl), 40-mins (Exposure), and 10-mins recovery (Post-Exp). Together with frontal hemodynamics, heart rate, objective and subjective vigilance, and self-evaluation of subjective symptoms were also assessed. The fNIRS results showed a slight influence of the GSM signal on frontal cortex, with a linear increase in [HHb] as a function of time in the Real exposure condition (F(4,40)=2.67; P=0.04). No other measure showed any GSM exposure-dependent changes. These results suggest that fNIRS is a convenient tool for safely and noninvasively investigating the cortical activation in MP exposure experimental settings. Given the short-term effects observed in this study, the results should be confirmed on a larger sample size and using a multichannel instrument that allows the investigation of a wider portion of the frontal cortex.

(NE) Curcio G, Nardo D, Perrucci MG, Pasqualetti P, Chen TL, Del Gratta C, Romani GL, Rossini PM. Effects of mobile phone signals over BOLD response while performing a cognitive task. Clin Neurophysiol. 123(1):129-136, 2012. (HU, BE, PE)

OBJECTIVE: The aim of this study was to investigate the effects induced by an exposure to a GSM signal (Global System for Mobile Communication) on brain BOLD (blood-oxygen-level dependent) response, as well as its time course while performing a Go-NoGo task. **METHODS:** Participants were tested twice, once in presence of a "real" exposure to GSM radiofrequency signal and once under a "sham" exposure (placebo condition). BOLD response of active brain areas and reaction times (RTs) while performing the task were measured both before and after the exposure. **RESULTS:** RTs to the somatosensory task did not change as a function of exposure (real vs sham) to GSM signal. BOLD results revealed significant activations in inferior parietal lobule, insula, precentral and postcentral gyri associated with Go responses after both "real" and "sham" exposure, whereas no significant effects were observed in the ROI analysis. **CONCLUSIONS:** The present fMRI study did not detect any brain activity changes by mobile phones. Also RTs in a somatosensory task resulted unaffected. **SIGNIFICANCE:** No changes in BOLD response have been observed as a consequence of RF-EMFs exposure.

(E) Daniels WM, Pitout IL, Afullo TJ, Mabandla MV. The effect of electromagnetic radiation in the mobile phone range on the behaviour of the rat. Metab Brain Dis. 24(4):629-641, 2009. (AS, ME, BE)

Electromagnetic radiation (EMR) is emitted from electromagnetic fields that surround power lines, household appliances and mobile phones. Research has shown that there are connections between EMR exposure and cancer and also that exposure to EMR may result in structural damage to neurons. In a study by Salford et al. (Environ Health Perspect 111:881-883, 2003) the authors demonstrated the presence of strongly stained areas in the brains of rats that were exposed to mobile phone EMR. These darker neurons were particularly prevalent in the hippocampal area of the brain. The aim of our study was to further investigate the effects of EMR. Since the hippocampus is involved in learning and memory and emotional states, we hypothesised that EMR will have a negative impact on the subject's mood and ability to learn. We subsequently performed behavioural, histological and biochemical tests on exposed and unexposed male and female rats to determine the effects of EMR on learning and memory, emotional states and corticosterone levels. We found no significant differences in the spatial memory test, and morphological assessment of the brain also yielded non-significant differences between the groups. However, in some exposed animals there were decreased locomotor activity, increased grooming and a tendency of increased basal corticosterone levels. These findings suggested that EMR exposure may lead to abnormal brain functioning.

***(NE) Danker-Hopfe H, Dorn H, Bornkessel C, Sauter C. Do mobile phone base stations affect sleep of residents? Results from an experimental double-blind sham-controlled field study. Am J Hum Biol. 22(5):613-618, 2010. (HU, BE, LI, SL) (*Effects observed probably not caused by exposure to RFR.)**

OBJECTIVES: The aim of the present double-blind, sham-controlled, balanced randomized cross-over study was to disentangle effects of electromagnetic fields (EMF) and non-EMF effects of mobile phone base stations on objective and subjective sleep quality. METHODS: In total 397 residents aged 18-81 years (50.9% female) from 10 German sites, where no mobile phone service was available, were exposed to sham and GSM (Global System for Mobile Communications, 900 MHz and 1,800 MHz) base station signals by an experimental base station while their sleep was monitored at their homes during 12 nights. Participants were randomly exposed to real (GSM) or sham exposure for five nights each. Individual measurement of EMF exposure, questionnaires on sleep disorders, overall sleep quality, attitude towards mobile communication, and on subjective sleep quality (morning and evening protocols) as well as objective sleep data (frontal EEG and EOG recordings) were gathered. RESULTS: Analysis of the subjective and objective sleep data did not reveal any significant differences between the real and sham condition. During sham exposure nights, objective and subjective sleep efficiency, wake after sleep onset, and subjective sleep latency were significantly worse in participants with concerns about possible health risks resulting from base stations than in participants who were not concerned. CONCLUSIONS: The study did not provide any evidence for short-term physiological effects of EMF emitted by mobile phone base stations on objective and subjective sleep quality. However, the results indicate that mobile phone base stations as such (not the electromagnetic fields) may have a significant negative impact on sleep quality.

(NE) Danker-Hopfe H, Dorn H, Bahr A, Anderer P, Sauter C. Effects of electromagnetic fields emitted by mobile phones (GSM 900 and WCDMA/UMTS) on the macrostructure of sleep. J Sleep Res. 20(1 Pt 1):73-81, 2011. (HU, BE, SL)

In the present double-blind, randomized, sham-controlled cross-over study, possible effects of electromagnetic fields emitted by Global System for Mobile Communications (GSM) 900 and Wideband Code-Division Multiple Access (WCDMA)/Universal Mobile Telecommunications System (UMTS) cell-phones on the macrostructure of sleep were investigated in a laboratory environment. An adaptation night, which served as screening night for sleep disorders and as an adjustment night to the laboratory environment, was followed by 9 study nights (separated by a 2-week interval) in which subjects were exposed to three exposure conditions (sham, GSM 900 and WCDMA/UMTS). The sample comprised 30 healthy male subjects within the age range 18-30 years (mean ± standard deviation: 25.3 ± 2.6 years). A cell-phone usage at maximum radio frequency (RF) output power was simulated and the transmitted power was adjusted in order to approach, but not to exceed, the specific absorption rate (SAR) limits of the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines for general public exposure (SAR(10g) = 2.0 W kg(-1)). In this study, possible effects of long-term (8 h) continuous RF exposure on the central nervous system were analysed during sleep, because sleep is a state in which many confounding intrinsic and extrinsic factors (e.g. motivation, personality, attitude) are eliminated or controlled. Thirteen of 177 variables characterizing the initiation and maintenance of sleep in the GSM 900 and three in the WCDMA exposure condition differed from the sham condition. The few significant results are not indicative of a negative impact on sleep architecture. From the present results there is no evidence for a sleep-disturbing effect of GSM 900 and WCDMA exposure.

(E) Dasdag S, Akdag MZ, Ulukaya E, Uzunlar AK, Ocak AR. Effect of mobile phone exposure on apoptotic glial cells and status of oxidative stress in rat brain. Electromagn Biol Med. 28(4):342-354, 2009. (AS, CE, CC, OX)

The aim of this study was to investigate the effects of mobile phone exposure on glial cells in brain. The study carried out on 31 Wistar Albino adult male rats. The rat heads in a carousel exposed to 900 MHz microwave. For the study group (n:14), rats exposed to the radiation 2 h per day (7 days in a week) for 10 months. For the sham group (n:7), rats were placed into the carousel and the same procedure was applied except that the generator was turned off. For the cage control (n:10), nothing applied to rats in this group. In this study, rats were euthanized after 10 months of exposure periods and brains were removed. Brain tissues were immunohistochemically stained for the active (cleaved) caspase-3, which is a well-known apoptosis marker, and p53. The expression of the proteins was evaluated by a semi-quantitative scoring system. However, total antioxidative capacity (TAC), catalase, total oxidant status (TOS), and oxidative stress index were measured in rat brain. Final score for apoptosis in the exposed group was significantly lower than the sham ($p < 0.001$) and the cage control groups ($p < 0.01$). p53 was not significantly changed by the exposure ($p > 0.05$). The total antioxidant capacity and catalase in the experimental group was found higher than that in the sham group ($p < 0.001$, $p < 0.05$). In terms of the TOS and oxidative stress index, there was no statistically significant difference between exposure and sham groups ($p > 0.05$). In conclusion, the final score for apoptosis, total antioxidant capacity and catalase in rat brain might be altered by 900 MHz radiation produced by a generator to represent exposure of global systems for mobile communication (GSM) cellular phones.

(E) Dasdag S, Akdag MZ, Kizil G, Kizil M, Cakir DU, Yokus B. Effect of 900 MHz radio frequency radiation on beta amyloid protein, protein carbonyl, and malondialdehyde in the brain. Electromagn Biol Med. 31(1):67-74, 2012. (AS, CE, CH, OX)

Recently, many studies have been carried out in relation to 900 MHz radiofrequency radiation (RF) emitted from a mobile phone on the brain. However, there is little data concerning possible mechanisms between long-term exposure of RF radiation and biomolecules in brain. Therefore, we aimed to investigate long-term effects of 900 MHz radiofrequency radiation on beta amyloid protein, protein carbonyl, and malondialdehyde in the rat brain. The study was carried out on 17 Wistar Albino adult male rats. The rat heads in a carousel were exposed to 900 MHz radiofrequency radiation emitted from a generator, simulating mobile phones. For the study group (n: 10), rats were exposed to the radiation 2 h per day (7 days a week) for 10 months. For the sham group (n: 7), rats were placed into the carousel and the same procedure was applied except that the generator was turned off. In this study, rats were euthanized after 10 months of exposure and their brains were removed. Beta amyloid protein, protein carbonyl, and malondialdehyde levels were found to be higher in the brain of rats exposed to 900 MHz radiofrequency radiation. However, only the increase of protein carbonyl in the brain of rats exposed to 900 MHz radiofrequency radiation was found to be statistically significant ($p<0.001$). In conclusion, 900 MHz radiation emitted from mobile/cellular phones can be an agent to alter some biomolecules such as protein. However, further studies are necessary.

(NE) de Gannes FP, Billaudel B, Taxile M, Haro E, Ruffié G, Lévêque P, Veyret B, Lagroye I. Effects of head-only exposure of rats to GSM-900 on blood-brain barrier permeability and neuronal degeneration. Radiat Res. 172(3):359-367, 2009. (AS, CE, ME, CC)

Salford et al. reported in 2003 that a single 2-h exposure to GSM-900 mobile telephony signals induced brain damage (increased permeability of the blood-brain barrier and presence of dark neurons) 50 days after exposure. In our study, 16 Fischer 344 rats (14 weeks old) were exposed head-only to the GSM-900 signal for 2 h at various brain-averaged SARs (0, 0.14 and 2.0 W/kg) or were used as cage or positive controls. Albumin leakage and neuron degeneration were evaluated 14 and 50 days after exposure. No apoptotic neurons were found 14 days after the last exposure using the TUNEL method. No statistically significant albumin leakage was observed. Neuronal degeneration, assessed using cresyl violet or the more specific marker Fluoro-Jade B, was not significantly different among the tested groups. No apoptotic neurons were detected. The findings of our study did not confirm the previous results of Salford et al.

(E) de Tommaso M, Rossi P, Falsaperla R, Francesco Vde V, Santoro R, Federici A. Mobile phones exposure induces changes of contingent negative variation in humans. Neurosci Lett. 464(2):79-83, 2009. (HU, EE)

Event-related potentials have been largely employed to test effects of GSM emissions on human brain. The aim of the present study was the evaluation of initial contingent negative variation (iCNV) changes, induced by 900 MHz GSM exposure, in a double blind design in healthy volunteers, subjected to a threefold experimental condition, EXPOSED (A), a real GSM phone emitting electromagnetic power, SHAM (B), a real phone where the electromagnetic power was dissipated on an internal load and OFF (C), a phone completely switched-off. Ten healthy right-handed volunteers were evaluated. The CNV was recorded during a 10 min time interval in each of the three experimental conditions A, B, and C, in order to assess the iCNV amplitude and habituation. The iCNV amplitude decreased and habituation increased during both A and B conditions, compared with condition C. This effect was diffuse over the scalp, and there was no significant prevalence of iCNV amplitude reduction on the left side, were the phones were located. Mobile Phones exposures A and B seemed to act on brain electrical activity, reducing the arousal and expectation of warning stimulus. This evidence, limited by the low number of subjects investigated, could be explained in terms of an effect induced by both the GSM signal and the extremely low frequency magnetic field produced by battery and internal circuits.

(E) Del Vecchio G, Giuliani A, Fernandez M, Mesirca P, Bersani F, Pinto R, Ardoino L, Lovisolo GA, Giardino L, Calzà L. Effect of radiofrequency electromagnetic field exposure on in vitro models of neurodegenerative disease. Bioelectromagnetics. 30(7):564-572, 2009. (CS, CE, IA, OX)

In this work we tested viability, proliferation, and vulnerability of neural cells, after continuous radiofrequency (RF) electromagnetic fields exposure (global system for mobile telecommunications (GSM) modulated 900 MHz signal at a specific absorption rate (SAR) of 1 W/kg and maximum duration 144 h) generated by transverse electromagnetic cells. We used two cellular systems, SN56 cholinergic for example, SN56 cholinergic cell line and rat primary cortical neurons, and well-known neurotoxic challenges, such as glutamate, 25-35AA beta-amyloid, and hydrogen peroxide. Exposure to RF did not change viability/proliferation rate of the SN56 cholinergic cells or viability of cortical neurons. Co-exposure to RF exacerbated neurotoxic effect of hydrogen peroxide in SN56, but not in primary cortical neurons, whereas no cooperative effects of RF with glutamate and 25-35AA beta-amyloid were found. These data suggest that only under particular circumstances exposure to GSM modulated, 900 MHz signal act as a co-stressor for oxidative damage of neural cells.

(E) Del Vecchio G, Giuliani A, Fernandez M, Mesirca P, Bersani F, Pinto R, Ardoino L, Lovisolo GA, Giardino L, Calzà L. Continuous exposure to 900MHz GSM-modulated EMF alters morphological maturation of neural cells. Neurosci Lett. 455(3):173-177, 2009. (CS, ME, DE)

The effects of radiofrequency electromagnetic field (RF-EMF) exposure on neuronal phenotype maturation have been studied in two different in vitro models: murine SN56 cholinergic cell line and rat primary cortical neurons. The samples were exposed at a dose of 1W/kg at 900 MHz GSM modulated. The phenotype analysis was carried out at 48 and 72 h (24 and 48 h of SN56 cell line differentiation) or at 24, 72, 120 h (2, 4 and 6 days in vitro for cortical neurons) of exposure, on live and immunolabeled neurons, and included the morphological study of neurite emission, outgrowth and branching. Moreover, cortical neurons were studied to detect alterations in the expression pattern of cytoskeleton regulating factors, e.g. beta-thymosin, and of early genes, e.g. c-Fos and c-Jun through real-time PCR on mRNA extracted after 24h exposure to EMF. We found that RF-EMF exposure reduced the number of neurites generated by both cell systems, and this alteration correlates to increased expression of beta-thymosin mRNA.

(E) Deshmukh PS, Banerjee BD, Abegaonkar MP, Megha K, Ahmed RS, Tripathi AK, Mediratta PK. Effect of low level microwave radiation exposure on cognitive function and oxidative stress in rats. Indian J Biochem Biophys. 50(2):114-119, 2013a. (AS, LI, CE, BE, OX)

Use of wireless communicating devices is increasing at an exponential rate in present time and is raising serious concerns about possible adverse effects of microwave (MW) radiation emitted from these devices on human health. The present study aimed to evaluate the effects of 900 MHz MW radiation exposure on cognitive function and oxidative stress in blood of Fischer rats. Animals were divided into two groups (6 animals/group): Group I (MW-exposed) and Group II (Sham-exposed). Animals were subjected to MW exposure (Frequency 900 MHz; specific absorption rate 8.4738 x 10(-5) W/kg) in Gigahertz transverse electromagnetic cell (GTEM) for 30 days (2 h/day, 5 days/week). Subsequently, cognitive function and oxidative stress parameters were examined for each group. Results showed significant impairment in cognitive function and increase in oxidative stress, as evidenced by the increase in levels of MDA (a marker of lipid peroxidation) and protein carbonyl (a marker of protein oxidation) and unaltered GSH content in blood. Thus, the study demonstrated that low level MW radiation had significant effect on cognitive function and was also capable of leading to oxidative stress.

(E) Deshmukh PS, Megha K, Banerjee BD, Ahmed RS, Chandna S, Abegaonkar MP, Tripathi AK. Detection of Low Level Microwave Radiation Induced Deoxyribonucleic Acid Damage Vis-à-vis Genotoxicity in Brain of Fischer Rats. Toxicol Int. 20(1):19-24, 2013b. (AS, LI, CE, CH)

BACKGROUND: Non-ionizing radiofrequency radiation has been increasingly used in industry, commerce, medicine and especially in mobile phone technology and has become a matter of serious concern in present time. OBJECTIVE: The present study was designed to investigate the possible deoxyribonucleic acid (DNA) damaging effects of low-level microwave radiation in brain of Fischer rats. MATERIALS AND METHODS: Experiments were performed on male Fischer rats exposed to microwave radiation for 30 days at three different frequencies: 900, 1800 and 2450 MHz. Animals were divided into 4 groups: Group I (Sham exposed): Animals not exposed to microwave radiation but kept under same conditions as that of other groups, Group II: Animals exposed to microwave radiation at frequency 900 MHz at specific absorption rate (SAR) 5.953 × 10(-4) W/kg, Group III: Animals exposed to 1800 MHz at SAR 5.835 × 10(-4) W/kg and Group IV: Animals exposed to 2450 MHz at SAR 6.672 × 10(-4) W/kg. At the end of the exposure period animals were sacrificed immediately and DNA damage in brain tissue was assessed using alkaline comet assay. RESULTS: In the present study, we demonstrated DNA damaging effects of low level microwave radiation in brain. CONCLUSION: We concluded that low SAR microwave radiation exposure at these frequencies may induce DNA strand breaks in brain tissue.

(E) Divan HA, Kheifets L, Obel C, Olsen J. Prenatal and postnatal exposure to cell phone use and behavioral problems in children. Epidemiology. 19(4):523-529, 2008. (HU, DE, BE)

BACKGROUND: The World Health Organization has emphasized the need for research into the possible effects of radiofrequency fields in children. We examined the association between prenatal and postnatal exposure to cell phones and behavioral problems in young children. METHODS: Mothers were recruited to the Danish National Birth Cohort early in pregnancy. When the children of those pregnancies reached 7 years of age in 2005 and 2006, mothers were asked to complete a questionnaire regarding the current health and behavioral status of children, as well as past exposure to cell phone use. Mothers evaluated the child's behavior problems using the Strength and Difficulties Questionnaire. RESULTS: Mothers of 13,159 children completed the follow-up questionnaire reporting their use of cell phones during pregnancy as well as current cell phone use by the child. Greater odds ratios for behavioral problems were observed for children who had possible prenatal or postnatal exposure to cell phone use. After adjustment for potential confounders, the odds ratio for a higher overall behavioral problems score was 1.80 (95% confidence interval = 1.45-2.23) in children with both prenatal and postnatal exposure to cell phones. CONCLUSIONS: Exposure to cell phones prenatally-and, to a lesser degree, postnatally-was associated with behavioral difficulties such as emotional and hyperactivity problems around the age of school entry. These associations may be noncausal and may be due to unmeasured confounding. If real, they would be of public health concern given the widespread use of this technology.

(NE) Divan HA, Kheifets L, Olsen J. Prenatal cell phone use and developmental milestone delays among infants. Scand J Work Environ Health. 37(4):341-348, 2011. (HU, DE, BE)

OBJECTIVE: The aim of this study was to examine if prenatal use of cell phones by pregnant mothers is associated with developmental milestones delays among offspring up to 18 months of age. **METHODS:** Our work is based upon the Danish National Birth Cohort (DNBC), which recruited pregnant mothers from 1996-2002, and was initiated to collect a variety of detailed information regarding in utero exposures and various health outcomes. At the end of 2008, over

41,000 singleton, live births had been followed with the Age-7 questionnaire, which collected cell phone use exposure for mothers during pregnancy. Outcomes for developmental milestones were obtained from telephone interviews completed by mothers at age 6 and 18 months postpartum. **RESULTS:** A logistic regression model estimated the odds ratios (OR) for developmental milestone delays, adjusted for potential confounders. Less than 5% of children at age 6 and 18 months had cognitive/language or motor developmental delays. At 6 months, the adjusted OR was 0.8 [95% confidence interval (95% CI) 0.7-1.0] for cognitive/language delay and 0.9 (95% CI 0.8-1.1) for motor development delay. At 18 months, the adjusted OR were 1.1 (95% CI 0.9-1.3) and 0.9 (95% CI 0.8-1.0) for cognitive/language and motor development delay, respectively. **CONCLUSIONS:** No evidence of an association between prenatal cell phone use and motor or cognitive/language developmental delays among infants at 6 and 18 months of age was observed. Even when considering dose-response associations for cell phone, associations were null.

(E) **Divan HA, Kheifets L, Obel C, Olsen J. Cell phone use and behavioural problems in young children. J Epidemiol Community Health. 66(6):524-529, 2012.** **(HU, DE, BE)**

BACKGROUND: Potential health effects of cell phone use in children have not been adequately examined. As children are using cell phones at earlier ages, research among this group has been identified as the highest priority by both national and international organisations. The authors previously reported results from the Danish National Birth Cohort (DNBC), which looked at prenatal and postnatal exposure to cell phone use and behavioural problems at age 7 years. Exposure to cell phones prenatally, and to a lesser degree postnatally, was associated with more behavioural difficulties. The original analysis included nearly 13 000 children who reached age 7 years by November 2006. **METHODS:** To see if a larger, separate group of DNBC children would produce similar results after considering additional confounders, children of mothers who might better represent current users of cell phones were analysed. This 'new' dataset consisted of 28 745 children with completed Age-7 Questionnaires to December 2008. **RESULTS:** The highest OR for behavioural problems were for children who had both prenatal and postnatal exposure to cell phones compared with children not exposed during either time period. The adjusted effect estimate was 1.5 (95% CI 1.4 to 1.7). **CONCLUSIONS:** The findings of the previous publication were replicated in this separate group of participants demonstrating that cell phone use was associated with behavioural problems at age 7 years in children, and this association was not limited to early users of the technology. Although weaker in the new dataset, even with further control for an extended set of potential confounders, the associations remained.

(NE) Dogan M, Turtay MG, Oguzturk H, Samdanci E, Turkoz Y, Tasdemir S, Alkan A, Bakir S. Effects of electromagnetic radiation produced by 3G mobile phones on rat brains: magnetic resonance spectroscopy, biochemical, and histopathological evaluation. Hum Exp Toxicol. 31(6):557-564, 2012. (AS, CE, OX, CC, CH)

Objective: The effects of electromagnetic radiation (EMR) produced by a third-generation (3G) mobile phone (MP) on rat brain tissues were investigated in terms of magnetic resonance spectroscopy (MRS), biochemistry, and histopathological evaluations. Methods: The rats were randomly assigned to two groups: Group 1 is composed of 3G-EMR-exposed rats (n = 9) and Group 2 is the control group (n = 9). The first group was subjected to EMR for 20 days. The control group was not exposed to EMR. Choline (Cho), creatinin (Cr), and N-acetylaspartate (NAA) levels were evaluated by MRS. Catalase (CAT) and glutathione peroxidase (GSH-Px) enzyme activities were measured by spectrophotometric method. Histopathological analyses were carried out to evaluate apoptosis in the brain tissues of both groups. Results: In MRS, NAA/Cr, Cho/Cr, and NAA/Cho ratios were not significantly different between Groups 1 and 2. Neither the oxidative stress parameters, CAT and GSH-Px, nor the number of apoptotic cells were significantly different between Groups 1 and 2. Conclusions: Usage of short-term 3G MP does not seem to have a harmful effect on rat brain tissue.

(E) Dragicevic N, Bradshaw PC, Mamcarz M, Lin X, Wang L, Cao C, Arendash GW. Long-term electromagnetic field treatment enhances brain mitochondrial function of both Alzheimer's transgenic mice and normal mice: a mechanism for electromagnetic field-induced cognitive benefit? Neuroscience 185:135-149, 2011. (AS, CE, CC, OX, MA)

We have recently reported that long-term exposure to high frequency electromagnetic field (EMF) treatment not only prevents or reverses cognitive impairment in Alzheimer's transgenic (Tg) mice, but also improves memory in normal mice. To elucidate the possible mechanism(s) for these EMF-induced cognitive benefits, brain mitochondrial function was evaluated in aged Tg mice and non-transgenic (NT) littermates following 1 month of daily EMF exposure. In Tg mice, EMF treatment enhanced brain mitochondrial function by 50-150% across six established measures, being greatest in cognitively-important brain areas (e.g. cerebral cortex and hippocampus). EMF treatment also increased brain mitochondrial function in normal aged mice, although the enhancement was not as robust and less widespread compared to that of Tg mice. The EMF-induced enhancement of brain mitochondrial function in Tg mice was accompanied by 5-10 fold increases in soluble Aβ1-40 within the same mitochondrial preparations. These increases in mitochondrial soluble amyloid-β peptide (Aβ) were apparently due to the ability of EMF treatment to disaggregate Aβ oligomers, which are believed to be the form of Aβ causative to mitochondrial dysfunction in Alzheimer's disease (AD). Finally, the EMF-induced mitochondrial enhancement in both Tg and normal mice occurred through non-thermal effects because brain temperatures were either stable or decreased during/after EMF treatment. These results collectively suggest that brain mitochondrial enhancement may be a primary mechanism through which EMF treatment provides cognitive benefit to both Tg and NT mice. Especially in the context that mitochondrial dysfunction is an early and prominent characteristic of Alzheimer's pathogenesis, EMF treatment could have profound value in the disease's prevention and treatment through intervention at the mitochondrial level.

(E) Eberhardt JL, Persson BR, Brun AE, Salford LG, Malmgren LO. Blood-brain barrier permeability and nerve cell damage in rat brain 14 and 28 days after exposure to microwaves from GSM mobile phones. Electromagn Biol Med. 27(3):215-229, 2008. (AS, ME, CC, LI)

We investigated the effects of global system for mobile communication (GSM) microwave exposure on the permeability of the blood-brain barrier and signs of neuronal damage in rats using a real GSM programmable mobile phone in the 900 MHz band. Ninety-six non-anaesthetized rats were either exposed to microwaves or sham exposed in TEM-cells for 2 h at specific absorption rates of average whole-body Specific Absorption Rates (SAR) of 0.12, 1.2, 12, or 120 mW/kg. The rats were sacrificed after a recovery time of either 14 or 28 d, following exposure and the extravazation of albumin, its uptake into neurons, and occurrence of damaged neurons was assessed. Albumin extravazation and also its uptake into neurons was seen to be enhanced after 14 d (Kruskal Wallis test: $p = 0.02$ and 0.002, respectively), but not after a 28 d recovery period. The occurrence of dark neurons in the rat brains, on the other hand, was enhanced later, after 28 d ($p = 0.02$). Furthermore, in the 28-d brain samples, neuronal albumin uptake was significantly correlated to occurrence of damaged neurons (Spearman $r = 0.41$; $p < 0.01$).

(NE) Eltiti S, Wallace D, Ridgewell A, Zougkou K, Russo R, Sepulveda F, Fox E. Short-term exposure to mobile phone base station signals does not affect cognitive functioning or physiological measures in individuals who report sensitivity to electromagnetic fields and controls. Bioelectromagnetics. 30(7):556-563, 2009. (HU, BE, LI)

Individuals who report sensitivity to electromagnetic fields often report cognitive impairments that they believe are due to exposure to mobile phone technology. Previous research in this area has revealed mixed results, however, with the majority of research only testing control individuals. Two studies using control and self-reported sensitive participants found inconsistent effects of mobile phone base stations on cognitive functioning. The aim of the present study was to clarify whether short-term (50 min) exposure at 10 mW/m(2) to typical Global System for Mobile Communication (GSM) and Universal Mobile Telecommunications System (UMTS) base station signals affects attention, memory, and physiological endpoints in sensitive and control participants. Data from 44 sensitive and 44 matched-control participants who performed the digit symbol substitution task (DSST), digit span task (DS), and a mental arithmetic task (MA), while being exposed to GSM, UMTS, and sham signals under double-blind conditions were analyzed. Overall, cognitive functioning was not affected by short-term exposure to either GSM or UMTS signals in the current study. Nor did exposure affect the physiological measurements of blood volume pulse (BVP), heart rate (HR), and skin conductance (SC) that were taken while participants performed the cognitive tasks.

(E) Eser O, Songur A, Aktas C, Karavelioglu E, Caglar V, Aylak F, Ozguner F, Kanter M. The effect of electromagnetic radiation on the rat brain: an experimental study. Turk Neurosurg. 23(6):707-715, 2013. (AS, CE, OX, ME)

AIM: The aim of this study is to determine the structural changes of electromagnetic waves in the frontal cortex, brain stem and cerebellum. MATERIAL and METHODS: 24 Wistar Albino adult male rats were randomly divided into four groups: group I consisted of control rats, and groups II-IV comprised electromagnetically irradiated (EMR) with 900, 1800 and 2450 MHz. The heads of the rats were exposed to 900, 1800 and 2450 MHz microwaves irradiation for 1h per day for 2 months. RESULTS: While the histopathological changes in the frontal cortex and brain stem were normal in the control group, there were severe degenerative changes, shrunken cytoplasm and extensively dark pyknotic nuclei in the EMR groups. Biochemical analysis demonstrated that the Total Antioxidative Capacity level was significantly decreased in the EMR groups and also Total Oxidative Capacity and Oxidative Stress Index levels were significantly increased in the frontal cortex, brain stem and cerebellum. IL-1β level was significantly increased in the EMR groups in the brain stem. CONCLUSION: EMR causes to structural changes in the frontal cortex, brain stem and cerebellum and impair the oxidative stress and inflammatory cytokine system. This deterioration can cause to disease including loss of these areas function and cancer development.

(E) Favre D. Mobile phone-induced honeybee worker piping Apidologie 42:270–279, 2011. (AS, BE)

The worldwide maintenance of the honeybee has major ecological, economic, and political implications. In the present study, electromagnetic waves originating from mobile phones were tested for potential effects on honeybee behavior. Mobile phone handsets were placed in the close vicinity of honeybees. The sound made by the bees was recorded and analyzed. The audiograms and spectrograms revealed that active mobile phone handsets have a dramatic impact on the behavior of the bees, namely by inducing the worker piping signal. In natural conditions, worker piping either announces the swarming process of the bee colony or is a signal of a disturbed bee colony.

(NE) Finnie JW, Blumbergs PC, Cai Z, Manavis J. Expression of the water channel protein, aquaporin-4, in mouse brains exposed to mobile telephone radiofrequency fields. Pathology. 41(5):473-475, 2009. (AS, CE, CC)

AIM: To determine whether exposure to mobile telephone radiofrequency (RF) fields, either acutely or long-term, produces up-regulation of the water channel protein, aquaporin-4 (AQP-4). **METHODS:** Using a purpose-designed exposure system at 900 MHz, mice were given a single, far-field whole body exposure at a specific absorption rate of 4 W/kg for 60 minutes or a similar exposure on 5 successive days/week for 104 weeks. Control mice were sham-exposed or freely mobile in a cage to control for any stress caused by restraint in the exposure module. A positive control group was given a clostridial toxin known to cause microvascular endothelial injury, severe vasogenic oedema and upregulation of AQP-4. Brains were perfusion fixed with 4% paraformaldehyde, coronal sections cut from six levels, and immunostained for the principal water channel protein in brain, AQP-4. **RESULTS:** There was no increase in AQP-4 expression in brains exposed to mobile phone microwaves compared to control (sham exposed and freely moving caged mice) brains after short or protracted exposure, while AQP-4 was substantially upregulated in the brains of mice given the clostridial toxin. **CONCLUSION:** Brains exposed to mobile telephone RF fields for a short (60 minutes) or long (2 years) duration did not show any immunohistochemically detectable up-regulation of the water channel protein, AQP-4, suggesting that there was no significant increase in blood-brain barrier permeability.

(NE) Finnie JW, Chidlow G, Blumbergs PC, Manavis J, Cai Z. Heat shock protein induction in fetal mouse brain as a measure of stress after whole of gestation exposure to mobile telephony radiofrequency fields. Pathology. 41(3):276-279, 2009. (AS, LE, CC, DE)

AIM: To determine whether whole of gestation exposure of fetal mouse brain to mobile telephone radiofrequency fields produces a stress response detectable by induction of heat shock proteins (HSPs). **METHODS:** Using a purpose-designed exposure system at 900 MHz, pregnant mice were given a single, far-field, whole body exposure at a specific absorption rate of 4 W/kg for 60 min/day from day 1 to day 19 of gestation. Control mice were sham-exposed or freely mobile in a cage to control for any stress caused by restraint in the exposure module. Immediately prior to parturition on day 19, fetal brains were collected, fixed in 4%

paraformaldehyde and paraffin-embedded. Three coronal sections encompassing a wide range of anatomical regions were cut from each brain and any stress response detected by immunostaining for HSP25, 32 and 70. **RESULTS:** There was no induction of HSP32 or 70 in any brains, while HSP25 expression was limited to two brainstem nuclei and occurred consistently in exposed and non-exposed brains. **CONCLUSION:** Whole of gestation exposure of fetal mouse brains to mobile phone radiofrequency fields did not produce any stress response using HSPs as an immunohistochemical marker.

(NE) Finnie JW, Cai Z, Manavis J, Helps S, Blumbergs PC. Microglial activation as a measure of stress in mouse brains exposed acutely (60 minutes) and long-term (2 years) to mobile telephone radiofrequency fields. Pathology. 42(2):151-154, 2010. (AS, CE, CC)

AIM: To determine whether acute or long-term exposure of the brain to mobile telephone radiofrequency (RF) fields produces activation of microglia, which normally respond rapidly to any change in their microenvironment. **METHODS:** Using a purpose designed exposure system at 900 MHz, mice were given a single, far-field whole body exposure at a specific absorption rate (SAR) of 4 W/kg for 60 min (acute) or on five successive days per week for 104 weeks (long-term). Control mice were sham-exposed or freely mobile in a cage to control for any stress caused by immobilisation in the exposure module. Positive control brains subjected to a stab wound were also included to confirm the ability of microglia to react to any neural stress. Brains were perfusion-fixed with 4% paraformaldehyde and representative regions of the cerebral cortex and hippocampus immunostained for ionised calcium binding adaptor molecule (Iba1), a specific microglial marker. **RESULTS:** There was no increase in microglial Iba1 expression in brains short or long-term exposed to mobile telephony microwaves compared to control (sham-exposed or freely moving caged mice) brains, while substantial microglial activation occurred in damaged positive control neural tissue. **CONCLUSION:** Acute (60 minutes) or longer duration (2 years) exposure of murine brains to mobile telephone RF fields did not produce any microglial activation detectable by Iba1 immunostaining.

(E) Fragopoulou AF, Miltiadous P, Stamatakis A, Stylianopoulou F, Koussoulakos SL, Margaritis LH. Whole body exposure with GSM 900MHz affects spatial memory in mice. Pathophysiology. 17(3):179-187, 2010. (AS, BE)

Extended work has been performed worldwide on the effects of mobile phone radiation upon rats' cognitive functions, however there is great controversy to the existence or not of deficits. The present work has been designed in order to test the effects of mobile phone radiation on spatial learning and memory in mice Mus musculus Balb/c using the Morris water maze (a hippocampal-dependent spatial memory task), since there is just one other study on mice with very low SAR level (0.05W/kg) showing no effects. We have applied a 2h daily dose of pulsed GSM 900MHz radiation from commercially available mobile phone for 4 days at SAR values ranging from 0.41 to 0.98W/kg. Statistical analysis revealed that during learning, exposed animals showed a deficit in transferring the acquired spatial information across training days (increased escape latency and distance swam, compared to the sham-exposed animals, on the first trial of training days 2-4). Moreover, during the memory probe-trial sham-exposed animals showed the expected preference for the target quadrant, while the exposed animals showed no preference, indicating that the exposed mice had deficits in consolidation and/or retrieval of the learned spatial information. Our results provide a basis for more thorough investigations considering reports on non-thermal effects of electromagnetic fields (EMFs).

(E) Fragopoulou AF, Samara A, Antonelou MH, Xanthopoulou A, Papadopoulou A, Vougas K, Koutsogiannopoulou E, Anastasiadou E, Stravopodis DJ, Tsangaris GT, Margaritis LH. Brain proteome response following whole body exposure of mice to mobile phone or wireless DECT base radiation. Electromagn Biol Med. 31(4):250-274, 2012. (AS, CE, CH, LI)

The objective of this study was to investigate the effects of two sources of electromagnetic fields (EMFs) on the proteome of cerebellum, hippocampus, and frontal lobe in Balb/c mice following long-term whole body irradiation. Three equally divided groups of animals (6 animals/group) were used; the first group was exposed to a typical mobile phone, at a SAR level range of 0.17-0.37 W/kg for 3 h daily for 8 months, the second group was exposed to a wireless DECT base (Digital Enhanced Cordless Telecommunications/Telephone) at a SAR level range of 0.012-0.028 W/kg for 8 h/day also for 8 months and the third group comprised the sham-exposed animals. Comparative proteomics analysis revealed that long-term irradiation from both EMF sources altered significantly ($p < 0.05$) the expression of 143 proteins in total (as low as 0.003 fold downregulation up to 114 fold overexpression). Several neural function related proteins (i.e., Glial Fibrillary Acidic Protein (GFAP), Alpha-synuclein, Glia Maturation Factor beta (GMF), and apolipoprotein E (apoE)), heat shock proteins, and cytoskeletal proteins (i.e., Neurofilaments and tropomodulin) are included in this list as well as proteins of the brain metabolism (i.e., Aspartate aminotransferase, Glutamate dehydrogenase) to nearly all brain regions studied. Western blot analysis on selected proteins confirmed the proteomics data. The observed protein expression changes may be related to brain plasticity alterations, indicative of oxidative stress in the nervous system or involved in apoptosis and might potentially explain human health hazards reported so far, such as headaches, sleep disturbance, fatigue, memory deficits, and brain tumor long-term induction under similar exposure conditions.

(NE) Fritzer G, Göder R, Friege L, Wachter J, Hansen V, Hinze-Selch D, Aldenhoff JB. Effects of short- and long-term pulsed radiofrequency electromagnetic fields on night sleep and cognitive functions in healthy subjects. Bioelectromagnetics. 28(4):316-325, 2007. (HU, BE, EE, SL)

There has been wide public discussion on whether the electromagnetic fields of mobile telephones and their base stations affect human sleep or cognitive functioning. As there is evidence for learning and memory-consolidating effects of sleep and particularly of REM sleep, disturbance of sleep by radiofrequency electromagnetic fields might also impair cognitive functions. Previously realized sleep studies yielded inconsistent results regarding short-term exposure. Moreover, data are lacking on the effect that short- and long-term exposure might have on sleep as well as on cognitive functions. Therefore, 10 healthy young male subjects were included and nocturnal sleep was recorded during eight consecutive nights. In the second, third, and last night, we investigated polysomnographic night sleep and cognitive functions. After the adaptation and baseline nights, the participants were exposed to a defined radiofrequency electromagnetic field during the following six nights. We analyzed polysomnographic night sleep according to Rechtschaffen and Kales [1968, Manual of Standardized Terminology, Techniques and Scoring System for Sleep of Human Subjects] as well as by power spectra and correlation dimension. Cognitive functions were investigated by an array of neuropsychological tests. Data analysis was done by comparing the baseline night with the first and last exposure night and the first two sleep cycles of the respective nights. We did not find significant effects, either on conventional sleep parameters or on power spectra and correlation dimension, nor were there any significant effects on cognitive functions. With our results, we are unable to reveal either short-term or cumulative long-term effects of radiofrequency electromagnetic fields on night sleep and cognitive functions in healthy young male subjects.

(E) Gao X, Luo R, Ma B, Wang H, Liu T, Zhang J, Lian Z, Cui X. [Interference of vitamin E on the brain tissue damage by electromagnetic radiation of cell phone in pregnant and fetal rats]. Wei Sheng Yan Jiu. 42(4):642-646, 2013.[Article in Chinese] **(AS, CE, ME, OX, DE)**

OBJECTIVE: To investigate the interference ot vitamin E on brain tissue damage by electromagnetic radiation of cell phone in pregnant and fetal rats. METHODS: 40 pregnant rats were randomly divided into five groups (positive control, negative control, low, middle and high dosage of vitamin E groups). The low, middle and high dosage of vitamin E groups were supplemented with 5, 15 and 30 mg/ml vitamin E respectively since the first day of pregnancy. And the negative control group and the positive control group were given peanut oil without vitamin E. All groups except for the negative control group were exposed to 900MHz intensity of cell phone radiation for one hour each time, three times per day for 21 days. After accouchement, the right hippocampus tissue of fetal rats in each group was taken and observed under electron microscope. The vitality of superoxide dismutase (SOD) and glutathione peroxidase (GSH-Px), and the content of malondialdehyde (MDA) in pregnant and fetal rats' brain tissue were tested. RESULTS: Compared with the negative control group, the chondriosomes in neuron and neuroglia of brain tissues was swelling, mild edema was found around the capillary, chromatin was concentrated and collected, and bubbles were formed in vascular endothelial cells (VEC) in the positive fetal rat control group, whereas the above phenomenon was un-conspicuous in the middle and high dosage of vitamin E groups. We can see uniform chromatin, abundant mitochondrion, rough endoplasmic reticulum and free ribosomes in the high dosage group. The apoptosis has not fond in all groups'sections. In the antioxidase activity analysis, compared with the negative control group, the vitality of SOD and GSH-Px significantly decreased and the content of MDA significantly increased both in the pregnant and fetal rats positive control group ($P < 0.05$). In fetal rats, the vitality of SOD and GSH-Px significantly increased in the brain tissues of all three different vitamin E dosages groups when compared with the positive control group, and the content of MDA was found significantly decreased in both middle and high dosage of vitamin E groups($P < 0.05$). The same results have also been found in high dosage pregnant rat group, but in middle dosage group only SOD activity was found increased with significance ($P < 0.05$). With the dosage increase of vitamin E, the vitality of SOD and GSH-Px was increasing and the content of MDA was decreasing. CONCLUSION: Under the experimental dosage, vitamin E has certain interference on damage of antioxidant capacity and energy metabolization induced by electromagnetic radiation of cell phone in pregnant rats and fetal rats.

(NE) Grafström G, Nittby H, Brun A, Malmgren L, Persson BR, Salford LG, Eberhardt J. Histopathological examinations of rat brains after long-term exposure to GSM-900 mobile phone radiation. Brain Res Bull. 77(5):257-263, 2008. **(AS, CE, ME, CH, LI)**

In order to mimic the real life situation, with often life-long exposure to the electromagnetic fields emitted by mobile phones, we have investigated in a rat model the effects of repeated exposures under a long period to Global System for Mobile Communication-900 MHz (GSM-900) radiation. Out of a total of 56 rats, 32 were exposed once weekly in a 2-h period, for totally 55 weeks, at different average whole-body specific absorption rates (SAR) (of in average 0.6 and 60 mW/kg at the initiation of the experimental period). The animals were exposed in a transverse electromagnetic transmission line chamber (TEM-cell) to radiation emitted by a GSM-900 test phone. Sixteen animals were sham exposed and eight animals were cage controls, which never left the animal house. After behavioural tests, 5-7 weeks after the last exposure, the brains were evaluated for histopathological alterations such as albumin extravasation, dark neurons, lipofuscin aggregation and signs of cytoskeletal and neuritic neuronal changes of the type seen in human ageing. In this study, no significant alteration of any these histopathological parameters was found, when comparing the GSM exposed animals to the sham exposed controls.

(NE) Guxens M, van Eijsden M, Vermeulen R, Loomans E, Vrijkotte TG, Komhout H, van Strien RT, Huss A. Maternal cell phone and cordless phone use during pregnancy and behaviour problems in 5-year-old children. J Epidemiol Community Health. 2013 Feb 5. [Epub ahead of print] (HU, DE, BE)

BACKGROUND: A previous study found an association between maternal cell phone use during pregnancy and maternal-reported child behaviour problems at age 7. Together with cell phones, cordless phones represent the main exposure source of radiofrequency-electromagnetic fields to the head. Therefore, we assessed the association between maternal cell phone and cordless phone use during pregnancy and teacher-reported and maternal-reported child behaviour problems at age 5. **METHODS:** The study was embedded in the Amsterdam Born Children and their Development study, a population-based birth cohort study in Amsterdam, the Netherlands (2003-2004). Teachers and mothers reported child behaviour problems using the Strength and Difficulties Questionnaire at age 5. Maternal cell phone and cordless phone use during pregnancy was asked when children were 7 years old. **RESULTS:** A total of 2618 children were included. As compared to non-users, those exposed to prenatal cell phone use showed an increased but non-significant association of having teacher-reported overall behaviour problems, although without dose-response relationship with the number of calls (OR=2.12 (95% CI 0.95 to 4.74) for <1 call/day, OR=1.58 (95% CI 0.69 to 3.60) for 1-4 calls/day and OR=2.04 (95% CI 0.86 to 4.80) for ≥5 calls/day). ORs for having teacher-reported overall behaviour problems across categories of cordless phone use were below 1 or close to unity. Associations of maternal cell phone and cordless phone use with maternal-reported overall behaviour problems remained non-significant. Non-significant associations were found for the specific behaviour problem subscales. **CONCLUSION:** Our results do not suggest that maternal cell phone or cordless phone use during pregnancy increases the odds of behaviour problems in their children.

(NE) Haarala C, Takio F, Rintee T, Laine M, Koivisto M, Revonsuo A, Hämäläinen H. Pulsed and continuous wave mobile phone exposure over left versus right hemisphere: effects on human cognitive function. Bioelectromagnetics. 28(4):289-295, 2007. (HU, BE)

The possible effects of continuous wave (CW) and pulse modulated (PM) electromagnetic field (EMF) on human cognition was studied in 36 healthy male subjects. They performed cognitive tasks while exposed to CW, PM, and sham EMF. The subjects performed the same tasks twice during each session; once with left-sided and once with right-sided exposure. The EMF conditions were spread across three testing sessions, each session separated by 1 week. The exposed hemisphere, EMF condition, and test order were counterbalanced over all subjects. We employed a double-blind design: both the subject and the experimenter were unaware of the EMF condition. The EMF was created with a signal generator connected via amplifier to a dummy phone antenna, creating a power output distribution similar to the original commercial mobile phone. The EMF had either a continuous power output of 0.25 W (CW) or pulsed power output with a mean of 0.25 W. An additional control group of 16 healthy male volunteers performed the same tasks without any exposure equipment to see if mere presence of the equipment could have affected the subjects' performance. No effects were found between the different EMF conditions, separate hemisphere exposures, or between the control and experimental group. In conclusion, the current results indicate that normal mobile phones have no discernible effect on human cognitive function as measured by behavioral tests.

(E) Haghani M, Shabani M, Moazzami K. Maternal mobile phone exposure adversely affects the electrophysiological properties of Purkinje neurons in rat offspring. Neuroscience. 2013 Jul 29. pii: S0306-4522(13)00643-X. doi: 10.1016/j.neuroscience.2013.07.049. [Epub ahead of print] (AS, CE, EE, CC, DE) no behavioral effect.

Electromagnetic field (EMF) radiations emitted from mobile phones may cause structural damage to neurons. With the increased usage of mobile phones worldwide, concerns about their possible effects on the nervous system are rising. In the present study, we aimed to elucidate the possible effects of prenatal EMF exposure on the cerebellum of offspring Wistar rats. Rats in EMF group were exposed to 900 MHz Pulse-EMF irradiation for six hours per day during all gestation period. Ten offspring's per each group were evaluated for behavioral and electrophysiological evaluations. Cerebellum - related behavioral dysfunctions were analyzed using motor learning and cerebellum-dependent functional tasks (Accelerated Rotarod, Hanging and Open field tests). Whole cell- patch clamp recordings were used for electrophysiological evaluations. The results of the present study failed to show any behavioral abnormalities in rats exposed to chronic EMF radiation. However, whole cell patch clamp recordings revealed decreased neuronal excitability of Purkinje cells in rats exposed to EMF. The most prominent changes included afterhyperpolarization amplitude, spike frequency, half width and first spike latency. In conclusion, the results of the present study show that prenatal EMF exposure results in altered electrophysiological properties of Purkinje neurons. However, these changes may not be severe enough to alter the cerebellum-dependent functional tasks.

(E) Hao D, Yang L, Chen S, Tong J, Tian Y, Su B, Wu S, Zeng Y. Effects of long-term electromagnetic field exposure on spatial learning and memory in rats. Neurol Sci. 2012 Feb 24. [Epub ahead of print] (AS, CE, BE, CC, EE)

With the development of communications industry, mobile phone plays an important role in daily life. Whether or not the electromagnetic radiation emitted by mobile phone causes any adverse effects on brain function has become of a great concern. This paper investigated the effect of electromagnetic field on spatial learning and memory in rats. 32 trained Wistar rats were divided into two groups: exposure group and control group. The exposure group was exposed to 916 MHz, 10w/m2 mobile phone electromagnetic field (EMF) 6 h a day, 5 days a week, 10 weeks. The completion time, number of total errors and the neuron discharge signals were recorded while the rats were searching for food in an eight-arm radial maze at every weekend. The neuron signals of one exposed rat and one control rat in the maze were obtained by the implanted microelectrode arrays in their hippocampal regions. It can be seen that during the weeks 4-5 of the experiment, the average completion time and error rate of the exposure group were longer and larger than that of control group ($p < 0.05$). During the weeks 1-3 and 6-9, they were close to each other. The hippocampal neurons showed irregular firing patterns and more spikes with shorter interspike interval during the whole experiment period. It indicates that the 916 MHz EMF influence learning and memory in rats to some extent in a period during exposure, and the rats can adapt to long-term EMF exposure.

(E) Hardell L, Söderqvist F, Carlberg M, Zetterberg H, Mild KH. Exposure to wireless phone emissions and serum beta-trace protein. Int J Mol Med. 26(2):301-306, 2010. (HU, CH, SL)

The lipocalin type of prostaglandin D synthase or beta-trace protein is synthesized in the choroid plexus, lepto-meninges and oligodendrocytes of the central nervous system and is secreted into the cerebrospinal fluid. beta-trace protein is the key enzyme in the synthesis of prostaglandin D2, an endogenous sleep-promoting neurohormone in the brain. Electromagnetic fields (EMF) in the radio frequency (RF) range have in some studies been associated with disturbed sleep. We studied the concentration of beta-trace protein in blood in relation to emissions from wireless phones. This study included 62 persons aged 18-30 years. The concentration of beta-trace protein decreased with increasing number of years of use of a wireless phone yielding a negative beta coefficient = -0.32, 95% confidence interval -0.60 to -0.04. Also cumulative use in hours gave a negative beta coefficient, although not statistically significant. Of the 62 persons, 40 participated in an experimental study with 30 min exposure to an 890-MHz GSM signal. No statistically significant change of beta-trace protein was found. In a similar study of the remaining 22 participitants with no exposure, beta-trace protein increased significantly over time, probably due to a relaxed situation. EMF emissions may down-regulate the synthesis of beta-trace protein. This mechanism might be involved in sleep disturbances reported in persons exposed to RF fields. The results must be interpreted with caution since use of mobile and cordless phones were self-reported. Awareness of exposure condition in the experimental study may have influenced beta-trace protein concentrations.

(NE) Hareuveny R, Eliyahu I, Luria R, Meiran N, Margaliot M. Cognitive effects of cellular phones: a possible role of non-radiofrequency radiation factors. Bioelectromagnetics. 32(7):585-588, 2011. (See also: Luria et al., 2009) (HU, BE)

Some studies found that cognitive functions of human beings may be altered while exposed to radiofrequency radiation (RFR) emitted by cellular phones. In two recent studies, we have found that experiment duration and exposure side (i.e., phone's location--right or left) may have a major influence on the detection of such effects. In this brief follow-up experiment, 29 right-handed male subjects were divided into two groups. Each subject had two standard cellular phones attached to both sides of his head. The subjects performed a spatial working memory task that required either a left-hand or a right-hand response under one of the two exposure conditions: left side of the head or right side. Contrary to our previous studies, in this work external antennas located far away from the subjects were connected to the cellular phones. This setup prevents any emission of RFR from the internal antenna, thus drastically reducing RFR exposure. Despite that, the results remain similar to those obtained in our previous work. These results indicate that some of the effects previously attributed to RFR can be the result of some confounders.

(NE) **Heinrich S, Thomas S, Heumann C, von Kries R, Radon K. Association between exposure to radiofrequency electromagnetic fields assessed by dosimetry and acute symptoms in children and adolescents: a population based cross-sectional study. Environ Health. 9:75, 2010. (HU, BE)**

BACKGROUND: The increase in numbers of mobile phone users was accompanied by some concern that exposure to radiofrequency electromagnetic fields (RF EMF) might adversely affect acute health especially in children and adolescents. The authors investigated this potential association using personal dosimeters. METHODS: A 24-hour exposure profile of 1484 children and 1508 adolescents was generated in a population-based cross-sectional study in Germany between 2006 and 2008 (participation 52%). Personal interview data on socio-demographic characteristics, self-reported exposure and potential confounders were collected. Acute symptoms were assessed twice during the study day using a symptom diary. RESULTS: Only few of the large number of investigated associations were found to be statistically significant. At noon, adolescents with a measured exposure in the highest quartile during morning hours reported a statistically significant higher intensity of headache (Odd Ratio: 1.50; 95% confidence interval: 1.03, 2.19). At bedtime, adolescents with a measured exposure in the highest quartile during afternoon hours reported a statistically significant higher intensity of irritation in the evening (4th quartile 1.79; 1.23, 2.61), while children reported a statistically significant higher intensity of concentration problems (4th quartile 1.55; 1.02, 2.33). CONCLUSIONS: We observed few statistically significant results which are not consistent over the two time points. Furthermore, when the 10% of the participants with the highest exposure are taken into consideration the significant results of the main analysis could not be confirmed. Based on the pattern of these results, we assume that the few observed significant associations are not causal but rather occurred by chance.

(NE) **Hirose H, Sakuma N, Kaji N, Nakayama K, Inoue K, Sekijima M, Nojima T, Miyakoshi J. Mobile phone base station-emitted radiation does not induce phosphorylation of Hsp27. Bioelectromagnetics. 28(2):99-108, 2007. (CS, CH, LI)**

An in vitro study focusing on the effects of low-level radiofrequency (RF) fields from mobile radio base stations employing the International Mobile Telecommunication 2000 (IMT-2000) cellular system was conducted to test the hypothesis that modulated RF fields act to induce phosphorylation and overexpression of heat shock protein hsp27. First, we evaluated the responses of human cells to microwave exposure at a specific absorption rate (SAR) of 80 mW/kg, which corresponds to the limit of the average whole-body SAR for general public exposure defined as a basic restriction in the International Commission on Non-Ionizing Radiation Protection (ICNIRP) guidelines. Second, we investigated whether continuous wave (CW) and Wideband Code Division Multiple Access (W-CDMA) modulated signal RF fields at 2.1425 GHz induced activation or gene expression of hsp27 and other heat shock proteins (hsps). Human glioblastoma A172 cells were exposed to W-CDMA radiation at SARs of 80 and 800 mW/kg for 2-48 h, and CW radiation at 80 mW/kg for 24 h. Human IMR-90 fibroblasts from fetal lungs were exposed to W-CDMA at 80 and 800 mW/kg for 2 or 28 h, and CW at 80 mW/kg for 28 h. Under the RF field exposure conditions described above, no significant differences in the expression levels of phosphorylated hsp27 at serine 82 (hsp27[pS82]) were observed between the test groups exposed to W-CDMA or CW signal and the sham-exposed negative controls, as evaluated immediately after the exposure periods by bead-based multiplex assays. Moreover, no noticeable differences in the gene expression of hsps were observed between the test groups and the negative controls by DNA Chip analysis. Our results confirm that exposure to low-level RF field up to 800 mW/kg does not induce phosphorylation of hsp27 or expression of hsp gene family.

(NE) Hirose H, Sasaki A, Ishii N, Sekijima M, Iyama T, Nojima T, Ugawa Y. 1950 MHz IMT-2000 field does not activate microglial cells in vitro. Bioelectromagnetics. 31(2):104-112, 2010. (CS, CC)

Given the widespread use of the cellular phone today, investigation of potential biological effects of radiofrequency (RF) fields has become increasingly important. In particular, much research has been conducted on RF effects on brain function. To examine any biological effects on the central nervous system (CNS) induced by 1950 MHz modulation signals, which are controlled by the International Mobile Telecommunication-2000 (IMT-2000) cellular system, we investigated the effect of RF fields on microglial cells in the brain. We assessed functional changes in microglial cells by examining changes in immune reaction-related molecule expression and cytokine production after exposure to a 1950 MHz Wideband Code Division Multiple Access (W-CDMA) RF field, at specific absorption rates (SARs) of 0.2, 0.8, and 2.0 W/kg. Primary microglial cell cultures prepared from neonatal rats were subjected to an RF or sham field for 2 h. Assay samples obtained 24 and 72 h after exposure were processed in a blind manner. Results showed that the percentage of cells positive for major histocompatibility complex (MHC) class II, which is the most common marker for activated microglial cells, was similar between cells exposed to W-CDMA radiation and sham-exposed controls. No statistically significant differences were observed between any of the RF field exposure groups and the sham-exposed controls in percentage of MHC class II positive cells. Further, no remarkable differences in the production of tumor necrosis factor-alpha (TNF-alpha), interleukin-1beta (IL-1beta), and interleukin-6 (IL-6) were observed between the test groups exposed to W-CDMA signal and the sham-exposed negative controls. These findings suggest that exposure to RF fields up to 2 W/kg does not activate microglial cells in vitro.

(E) Hountala CD, Maganioti AE, Papageorgiou CC, Nanou ED, Kyprianou MA, Tsiafakis VG, Rabavilas AD, Capsalis CN. The spectral power coherence of the EEG under different EMF conditions. Neurosci Lett. 441(2):188-192, 2008. (HU, EE)

The present study introduces the concept of spectral power coherence (SPC), which reflects the pattern of coordination of the four basic EEG bands (delta, theta, alpha, and beta) at a specific location of the brain. The SPC was calculated for the pre-stimulus EEG signal during an auditory memory task under different electromagnetic field (EMF) conditions (900 MHz and 1800 MHz). The results showed that delta rhythm is less consequential in the overall cooperation between the bands than the higher frequency theta, alpha and beta rhythms. Additionally, it has been shown that the radiation effect on SPC is different for the two genders. In the absence of radiation males exhibit higher overall SPC than females. These differences disappear in the presence of 900 MHz and are reversed in the presence of 1800 MHz.

(E) Hung CS, Anderson C, Horne JA, McEvoy P. Mobile phone 'talk-mode' signal delays EEG-determined sleep onset. Neurosci Lett. 421(1):82-86, 2007. (HU, EE, BE, WS, SL)

Mobile phones signals are pulse-modulated microwaves, and EEG studies suggest that the extremely low-frequency (ELF) pulse modulation has sleep effects. However, 'talk', 'listen' and 'standby' modes differ in the ELF (2, 8, and 217Hz) spectral components and specific absorption rates, but no sleep study has differentiated these modes. We used a GSM900 mobile phone controlled by a base-station simulator and a test SIM card to simulate these three specific modes, transmitted at 12.5% (23dBm) of maximum power. At weekly intervals, 10 healthy young adults, sleep restricted to 6h, were randomly and single-blind exposed to one of: talk, listen, standby and sham (nil signal) modes, for 30 min, at 13:30 h, whilst lying in a sound-proof, lit bedroom, with a thermally insulated silent phone beside the right ear. Bipolar EEGs were recorded continuously, and subjective ratings of sleepiness obtained every 3 min (before, during and after exposure). After exposure the phone and base-station were switched off, the bedroom darkened, and a 90 min sleep opportunity followed. We report on sleep onset using: (i) visually scored latency to onset of stage 2 sleep, (ii) EEG power spectral analysis. There was no condition effect for subjective sleepiness. Post-exposure, sleep latency after talk mode was markedly and significantly delayed beyond listen and sham modes. This condition effect over time was also quite evident in 1-4Hz EEG frontal power, which is a frequency range particularly sensitive to sleep onset. It is possible that 2, 8, 217Hz modulation may differentially affect sleep onset.

(E) İkinci A, Odacı E, Yıldırım M, Kaya H, Akça M, Hancı H, Aslan A, Sönmez OF, Baş O. The Effects of Prenatal Exposure to a 900 Megahertz Electromagnetic Field on Hippocampus Morphology and Learning Behavior in Rat Pups. NeuroQuantology. 11(4):582-590, 2013. (AS, BE, ME, CE, DE)

The purpose of this study was to examine the effect on hippocampus morphology and learning behavior in rat pups following prenatal exposure to a 900 megahertz (MHz) electromagnetic field (EMF). Female Sprague Dawley rats weighing 180-250 g were left to mate with males. The following day, pregnant rats identified as such by the vaginal smear test were divided into two groups, control (n=3) and EMF (n=3). No procedures were performed on the control group. The rats in the EMF group were exposed to 900 MHz EMF on days 13 to 21 of pregnancy, for 1 h a day. Female rat pups were removed from their mothers at 22 days old. We then established two newborn rat groups, a 13 member control group and a 10 member EMF group. Radial arm maze and passive avoidance tests were used to measure rat pups' learning and memory performance. All rats were decapitated on the postnatal 32nd day. Routine histological procedures were performed on the brain tissues, and sections were stained with Cresyl fast violet. The radial arm maze (p=0.007) and passive avoidance (p=0.032) tests were administered to both groups under identical conditions, and compromised learning behavior was determined in the EMF group rats. Morphological compromise was also determined in the EMF group sections. Our results show that the application of a 900 MHz EMF in the prenatal period adversely affected female pups' learning behavior and also resulted in histopathological changes appearing in the hippocampus.

(E) Imge EB, Kiliçoğlu B, Devrim E, Cetin R, Durak I. Effects of mobile phone use on brain tissue from the rat and a possible protective role of vitamin C - a preliminary study. Int J Radiat Biol. 86(12):1044-1049, 2010. (AS, CE, CH, OX)

PURPOSE: To evaluate effects of mobile phone use on brain tissue and a possible protective role of vitamin C. **MATERIALS AND METHODS:** Forty female rats were divided into four groups randomly (Control, mobile phone, mobile phone plus vitamin C and, vitamin C alone). The mobile phone group was exposed to a mobile phone signal (900 MHz), the mobile phone plus vitamin C group was exposed to a mobile phone signal (900 MHz) and treated with vitamin C administered orally (per os). The vitamin C group was also treated with vitamin C per os for four weeks. Then, the animals were sacrificed and brain tissues were dissected to be used in the analyses of malondialdehyde (MDA), antioxidant potential (AOP), superoxide dismutase, catalase (CAT), glutathione peroxidase (GSH-Px), xanthine oxidase, adenosine deaminase (ADA) and 5'nucleotidase (5'-NT). **RESULTS:** Mobile phone use caused an inhibition in 5'-NT and CAT activities as compared to the control group. GSH-Px activity and the MDA level were also found to be reduced in the mobile phone group but not significantly. Vitamin C caused a significant increase in the activity of GSH-Px and non-significant increase in the activities of 5'-NT, ADA and CAT enzymes. **CONCLUSION:** Our results suggest that vitamin C may play a protective role against detrimental effects of mobile phone radiation in brain tissue.

(NE) Inomata-Terada S, Okabe S, Arai N, Hanajima R, Terao Y, Frubayashi T, Ugawa Y. Effects of high frequency electromagnetic field (EMF) emitted by mobile phones on the human motor cortex. Bioelectromagnetics. 28(7):553-561, 2007. (HU, EE)

We investigated whether the pulsed high frequency electromagnetic field (EMF) emitted by a mobile phone has short term effects on the human motor cortex. We measured motor evoked potentials (MEPs) elicited by single pulse transcranial magnetic stimulation (TMS), before and after mobile phone exposure (active and sham) in 10 normal volunteers. Three sites were stimulated (motor cortex (CTX), brainstem (BST) and spinal nerve (Sp)). The short interval intracortical inhibition (SICI) of the motor cortex reflecting GABAergic interneuronal function was also studied by paired pulse TMS method. MEPs to single pulse TMS were also recorded in two patients with multiple sclerosis showing temperature dependent neurological symptoms (hot bath effect). Neither MEPs to single pulse TMS nor the SICI was affected by 30 min of EMF exposure from mobile phones or sham exposure. In two MS patients, mobile phone exposure had no effect on any parameters of MEPs even though conduction block occurred at the corticospinal tracts after taking a bath. As far as available methods are concerned, we did not detect any short-term effects of 30 min mobile phone exposure on the human motor cortical output neurons or interneurons even though we can not exclude the possibility that we failed to detect some mild effects due to a small sample size in the present study. This is the first study of MEPs after electromagnetic exposure from a mobile phone in neurological patients.

(NE) Irlenbusch L, Bartsch B, Cooper J, Herget I, Marx B, Raczek J, Thoss F. Influence of a 902.4 MHz GSM signal on the human visual system: investigation of the discrimination threshold. Bioelectromagnetics. 28(8):648-654, 2007. (HU, EE, LI)

The proximity of a mobile phone to the human eye raises the question as to whether radiofrequency (RF) electromagnetic fields (EMF) affect the visual system. A basic characteristic of the human eye is its light sensitivity, making the visual discrimination threshold (VDThr) a suitable parameter for the investigation of potential effects of RF exposure on the eye. The VDThr was measured for 33 subjects under standardized conditions. Each subject took part in two experiments (RF-exposure and sham-exposure experiment) on different days. In each experiment, the VDThr was measured continuously in time intervals of about 10 s for two periods of 30 min, having a break of 5 min in between. The sequence of the two experiments was randomized, and the study was single blinded. During the RF exposure, a GSM signal of 902.4 MHz (pulsed with 217 Hz) was applied to the subjects. The power flux density of the electromagnetic field at the subject location (in the absence of the subject) was 1 W/m(2), and numerical dosimetry calculations determined corresponding maximum local averaged specific absorption rate (SAR) values in the retina of SAR(1 g) = 0.007 W/kg and SAR(10 g) = 0.003 W/kg. No statistically significant differences in the VDThr were found in comparing the data obtained for RF exposure with those for sham exposure.

(E) Jing J, Yuhua Z, Xiao-qian Y, Rongping J, Dong-mei G, Xi C. The influence of microwave radiation from cellular phone on fetal rat brain. Electromagn Biol Med. 31(1):57-66, 2012. (AS, CE, CH, OX, DE)

The increasing use of cellular phones in our society has brought focus on the potential detrimental effects to human health by microwave radiation. The aim of our study was to evaluate the intensity of oxidative stress and the level of neurotransmitters in the brains of fetal rats chronically exposed to cellular phones. The experiment was performed on pregnant rats exposed to different intensities of microwave radiation from cellular phones. Thirty-two pregnant rats were randomly divided into four groups: CG, GL, GM, and GH. CG accepted no microwave radiation, GL group radiated 10 min each time, GM group radiated 30 min, and GH group radiated 60 min. The 3 experimental groups were radiated 3 times a day from the first pregnant day for consecutively 20 days, and on the 21st day, the fetal rats were taken and then the contents of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), malondialdehyde (MDA), noradrenaline (NE), dopamine (DA), and 5-hydroxyindole acetic acid (5-HT) in the brain were assayed. Compared with CG, there were significant differences ($P<0.05$) found in the contents of SOD, GSH-Px, and MDA in GM and GH; the contents of SOD and GSH-Px decreased and the content of MDA increased. The significant content differences of NE and DA were found in fetal rat brains in GL and GH groups, with the GL group increased and the GH group decreased. Through this study, we concluded that receiving a certain period of microwave radiation from cellular phones during pregnancy has certain harm on fetal rat brains.

(E) Jorge-Mora T, Folgueiras MA, Leiro-Vidal JM, Jorge-Barreiro FJ, Ares-Pena FJ, López-Martin E. Exposure to 2.45 GHz Microwave Radiation Provokes Cerebral Changes in Induction of Hsp-90 α/β Heat Shock Protein in Rat. Prog Electromagn Res, 100:351-379, 2010. (AS, CC, CH)

Physical agents such as non-ionizing continuous-wave 2.45 GHz radiation may cause damage that alters cellular homeostasis and may trigger activation of the genes that encode heat shock proteins (HSP). We used Enzyme-Linked ImmunoSorbent Assay (ELISA) and immunohistochemistry to analyze the changes in levels of HSP-90 and its distribution in the brain of Sprague-Dawley rats, ninety minutes and twenty-four hours after acute (30min) continuous exposure to 2.45 GHz radiation in a the Gigahertz Transverse Electromagnetic

(GTEM cell). In addition, we studied further indicators of neuronal insult: dark neurons, chromatin condensation and nucleus fragmentation, which were observed under optical conventional or fluorescence microscopy after DAPI staining. The cellular distribution of protein HSP-90 in the brain increased with each corresponding SAR ($0.034 \pm 3.10^{-3,}$ 0.069 ± 5.10^{-3}, 0.27 ± 21.10^{-3} W/kg), in hypothalamic nuclei, limbic cortex and somatosensorial cortex after exposure to the radiation. At twenty-four hours post-irradiation, levels of HSP-90 protein remained high in all hypothalamic nuclei for all SARs, and in the parietal cortex, except the limbic system, HSP-90 levels were lower than in non-irradiated rats, almost half the levels in rats exposed to the highest power radiation. Non-apoptotic cellular nuclei and some dark neurons were found ninety minutes and twenty-four hours after maximal SAR exposure. The results suggest that acute exposure to electromagnetic fields triggered an imbalance in anatomical HSP- 90 levels but the anti-apoptotic mechanism is probably sufficient to compensate the non-ionizing stimulus. Further studies are required to determine the regional effects of chronic electromagnetic pollution on heat shock proteins and their involvement in neurological processes and neuronal damage.

(NE) Joubert V, Leveque P, Cueille M, Bourthoumieu S, Yardin C. No apoptosis is induced in rat cortical neurons exposed to GSM phone fields. Bioelectromagnetics. 28(2):115-121, 2007. (CS, CC)

The aim of this study was to investigate the radiofrequency (RF) electromagnetic fields (EMF) effects on neuronal apoptosis in vitro. Primary cultured neurons from cortices of embryonic Wistar rats were exposed to a 900-MHz global system for mobile communication (GSM) RF field for 24 h in a wire-patch cell. The average-specific absorption rate (SAR) used was 0.25 W/kg. Apoptosis rate was assessed immediately or 24 h after exposure using three methods: (i) DAPI staining; (ii) flow cytometry using double staining with TdT-mediated dUTP nick-end labeling (TUNEL) and propidium iodide (PI); and (iii) measurement of caspase-3 activity by fluorimetry. No statistically significant difference in the apoptosis rate was observed between controls and 24 h GSM-exposed neurons, either 0 h or 24 h post-exposure. All three methods used to assess apoptosis were concordant. These results showed that, under the conditions of experiment used, GSM-exposure does not significantly increase the apoptosis rate in rat primary neuronal cultures. This work is in accordance with other studies performed on cell lines and, to our knowledge, is the first one performed on cultured cortical neurons.

****(E) Joubert, V., Bourthoumieu, S., Leveque, P. and Yardin, C. Apoptosis is Induced by Radiofrequency Fields through the Caspase-Independent Mitochondrial Pathway in Cortical Neurons. Radiat. Res. 169, 38-45, 2008. (CS, CC)**

In the present study, we investigated whether continuous-wave (CW) radiofrequency (RF) fields induce neuron apoptosis in vitro. Rat primary neuronal cultures were exposed to a CW 900 MHz RF field with a specific absorption rate (SAR) of 2 W/kg for 24 h. During exposure, an increase of 2 degrees C was measured in the medium; control experiments with neurons exposed to 39 degrees C were then performed. Apoptosis was assessed by condensation of nuclei with 4',6-diamino-2-phenylindole (DAPI) staining observed with an epifluorescence microscope and fragmentation of DNA with TdT-mediated dUTP nick-end labeling (TUNEL) analyzed by flow cytometry. A statistically significant difference in the rate of apoptosis was found in the RF-field-exposed neurons compared to the sham-, 37 degrees C- and 39 degrees C-exposed neurons either 0 or 24 h after exposure using both methods. To assess whether the observed apoptosis was caspase-dependent or -independent, assays measuring caspase 3 activity and apoptosis-inducing factor (AIF) labeling were performed. No increase in the caspase 3 activity was found, whereas the percentage of AIF-positive nuclei in RF-field-exposed neurons was increased by three- to sevenfold compared to other conditions. Our results show that, under the experimental conditions used, exposure of primary rat neurons to CW RF fields may induce a caspase-independent pathway to apoptosis that involves AIF.

(E) Júnior LC, Guimarães ED, Musso CM, Stabler CT, Garcia RM, Mourão-Júnior CA, Andreazzi AE. Behavior and memory evaluation of Wistar rats exposed to 1·8 GHz radiofrequency electromagnetic radiation. Neurol Res. 2014 Jan 27:1743132813Y0000000276. [Epub ahead of print] (AS, CE, BE)

Background: The development of communication systems has brought great social and economic benefits to society. As mobile phone use has become widespread, concerns have emerged regarding the potential adverse effects of radiofrequency electromagnetic radiation (RF-EMR) used by these devices. Objective: To verify potential effects of mobile phone radiation on the central nervous system (CNS) in an animal model. Methods: Male Wistar rats (60 days old) were exposed to RF-EMR from a Global System for Mobile (GSM) cell phone (1·8 GHz) for 3 days. At the end of the exposure, the following behavioral tests were performed: open field and object recognition. Results: Our results showed that exposed animals did not present anxiety patterns or working memory impairment, but stress behavior actions were observe. Conclusion: Given the results of the present study, we speculate that RF-EMR does not promote CNS impairment, but suggest that it may lead to stressful behavioral patterns.

(NE) Kang KA, Lee HC, Lee JJ, Hong MN, Park MJ, Lee YS, Choi HD, Kim N, Ko YK, Lee JS. Effects of combined radiofrequency radiation exposure on levels of reactive oxygen species in neuronal cells. J Radiat Res. 2013 Oct 8. [Epub ahead of print] (CS, OX, IA)

The objective of this study was to investigate the effects of the combined RF radiation (837 MHz CDMA plus 1950 MHz WCDMA) signal on levels of intracellular reactive oxygen species (ROS) in neuronal cells. Exposure of the combined RF signal was conducted at specific absorption rate values of 2 W/kg of CDMA plus 2 W/kg of WCDMA for 2 h. Co-exposure to combined RF radiation with either H2O2 or menadione was also performed. The experimental exposure groups were incubator control, sham-exposed, combined RF radiation-exposed with or without either H2O2 or menadione groups. The intracellular ROS level was measured by flow cytometry using the fluorescent probe dichlorofluorescein diacetate. Intracellular ROS levels were not consistently affected by combined RF radiation exposure alone in a time-dependent manner in U87, PC12 or SH-SY5Y cells. In neuronal cells exposed to combined RF radiation with either H2O2 or menadione, intracellular ROS levels showed no statically significant alteration compared with exposure to menadione or H2O2 alone. These findings indicate that neither combined RF radiation alone nor combined RF radiation with menadione or H2O2 influences the intracellular ROS level in neuronal cells such as U87, PC12 or SH-SY5Y.

(E) Kaprana AE, Chimona TS, Papadakis CE, Velegrakis SG, Vardiambasis IO, Adamidis G, Velegrakis GA. Auditory brainstem response changes during exposure to GSM-900 radiation: an experimental study. Audiol Neurootol. 16(4):270-276, 2011. (HU, EE)

The objective of the present study was to investigate the possible electrophysiological time-related changes in auditory pathway during mobile phone electromagnetic field exposure. Thirty healthy rabbits were enrolled in an experimental study of exposure to GSM-900 radiation for 60 min and auditory brainstem responses (ABRs) were recorded at regular time-intervals during exposure. The study subjects were radiated via an adjustable power and frequency radio transmitter for GSM-900 mobile phone emission simulation, designed and manufactured according to the needs of the experiment. The mean absolute latency of waves III-V showed a statistically significant delay ($p < 0.05$) after 60, 45 and 15 min of exposure to electromagnetic radiation of 900 MHz, respectively. Interwave latency I-III was found to be prolonged after 60 min of radiation exposure in correspondence to wave III absolute latency delay. Interwave latencies I-V and III-V were found with a statistically significant delay ($p < 0.05$) after 30 min of radiation. No statistically significant delay was found for the same ABR parameters in recordings from the ear contralateral to the radiation source at 60 min radiation exposure compared with baseline ABR. The ABR measurements returned to baseline recordings 24 h after the exposure to electromagnetic radiation of 900 MHz. The prolongation of interval latencies I-V and III-V indicates that exposure to electromagnetic fields emitted by mobile phone can affect the normal electrophysiological activity of the auditory system, and these findings fit the pattern of general responses to a stressor.

(E) Karaca E, Durmaz B, Aktug H, Yildiz T, Guducu C, Irgi M, Koksal MG, Ozkinay F, Gunduz C, Cogulu O. The genotoxic effect of radiofrequency waves on mouse brain. J Neurooncol. 106(1):53-58, 2012. (CS, CH)

Concerns about the health effects of radiofrequency (RF) waves have been raised because of the gradual increase in usage of cell phones, and there are scientific questions and debates about the safety of those instruments in daily life. The aim of this study is to evaluate the genotoxic effects of RF waves in an experimental brain cell culture model. Brain cell cultures of the mice were exposed to 10.715 GHz with specific absorbtion rate (SAR) 0.725 W/kG signals for 6 h in 3 days at 25°C to check for the changes in the micronucleus (MNi) assay and in the expression of 11 proapoptotic and antiapoptotic genes. It was found that MNi rate increased 11-fold and STAT3 expression decreased 7-fold in the cell cultures which were exposed to RF. Cell phones which spread RF may damage DNA and change gene expression in brain cells.

(E) Kesari KK, Kumar S, Behari J. 900-MHz microwave radiation promotes oxidation in rat brain. Electromagn Biol Med. 30(4):219-234, 2011. (AS, CE, CH, OX)

Recently, there have been several reports referring to detrimental effects due to radio frequency electromagnetic fields (RF-EMF) exposure. Special attention was given to investigate the effect of mobile phone exposure on the rat brain. Since the integrative mechanism of the entire body lies in the brain, it is suggestive to analyze its biochemical aspects. For this, 35-day old Wistar rats were exposed to a mobile phone for 2 h per day for a duration of 45 days where specific absorption rate (SAR) was 0.9 W/Kg. Animals were divided in two groups: sham exposed ($n = 6$) and exposed group ($n = 6$). Our observations indicate a significant decrease ($P < 0.05$) in the level of glutathione peroxidase, superoxide dismutase, and an increase in catalase activity. Moreover, protein kinase shows a significant decrease in exposed group ($P < 0.05$) of hippocampus and whole brain. Also, a significant decrease ($P < 0.05$) in the level of pineal melatonin and a significant increase ($P < 0.05$) in creatine kinase and caspase 3 was observed in exposed group of whole brain as compared with sham exposed. Finally, a significant increase in the level of ROS (reactive oxygen species) ($P < 0.05$) was also recorded. The study concludes that a reduction or an increase in antioxidative enzyme activities, protein kinase C, melatonin, caspase 3, and creatine kinase are related to overproduction of reactive oxygen species (ROS) in animals under mobile phone radiation exposure. Our findings on these biomarkers are clear indications of possible health implications.

(E) Khullar S1, Sood A2, Sood S3. Auditory Brainstem Responses and EMFs Generated by Mobile Phones. Indian J Otolaryngol Head Neck Surg. 65(Suppl 3):645-649, 2013. (HU, EE)

There has been a manifold increase in the number of mobile phone users throughout the world with the current number of users exceeding 2 billion. However this advancement in technology like many others is accompanied by a progressive increase in the frequency and intensity of electromagnetic waves without consideration of the health consequences. The aim of our study was to advance our understanding of the potential adverse effects of GSM mobile phones on auditory brainstem responses (ABRs). 60 subjects were selected for the study and divided into three groups of 20 each based on their usage of mobile phones. Their ABRs were recorded and analysed for latency of waves I-V as well as interpeak latencies I-III, I-V and III-V (in ms). Results revealed no significant difference in the ABR parameters between group A (control group) and group B (subjects using mobile phones for maximum 30 min/day for 5 years). However the latency of waves was significantly prolonged in group C (subjects using mobile phones for 10 years for a maximum of 30 min/day) as compared to the control group. Based on our findings we concluded that long term exposure to mobile phones may affect conduction in the peripheral portion of the auditory pathway. However more research needs to be done to study the long term effects of mobile phones particularly of newer technologies like smart phones and 3G.

(NE) Kim HS, An YS, Paik MJ, Lee YS, Choi HD, Kim BC, Pack JK, Kim N, Ahn YH. The effects of exposure to 915 MHz **radiofrequency identification on cerebral glucose metabolism in rat: A [F-18] FDG micro-PET study. Int J Radiat Biol. 2013 May 7. [Epub ahead of print] (AS, CE, CC, CH)**

Purpose: We investigated the effect of whole-body exposure to 915-MHz radiofrequency identification (RFID) on rat cortical glucose metabolism by using ^{18}F-deoxyglucose positron emission tomography (FDG-PET). Materials and methods: Male Sprague-Dawley rats were divided into three groups: Cage-control, sham-exposed and RFID-exposed groups. Rats were exposed to the 915-MHz RFID for 8 h daily, 5 days per week, for 2 or 16 weeks. The whole-body average specific absorption rate (SAR) was 4 W/kg for the field of the 915 MHz RFID signal. FDG-PET images were obtained the day after RFID exposure, using micro-PET with a FDG tracer. With a Xeleris functional imaging workstation, absolute values in regions of interest (ROI) in the frontal, temporal and parietal cortexes and cerebellum were measured. Cortical ROI values were normalized to the cerebellar value and compared. Results: The data showed that the relative cerebral glucose metabolic rate was unchanged in the frontal, temporal and parietal cortexes of the 915 MHz RFID-exposed rats, compared with rats in cage-control and sham-exposed groups. Conclusion: Our results suggest that 915 MHz RFID radiation exposure did not cause a significant long lasting effect on glucose metabolism in the rat brain.

(NE) Kim TH, Huang TQ, Jang JJ, Kim MH, Kim HJ, Lee JS, Pack JK, Seo JS, Park WY. Local exposure of 849 MHz and 1763 MHz radiofrequency radiation to mouse heads does not induce cell death or cell proliferation in brain. Exp Mol Med. 40(3):294-303, 2008. (AS, CE, CC) Erratum in: Exp Mol Med. 2008 Aug 31;40(4):477. Kim, Tae-Hyoung [corrected to Kim, Tae-Hyung].

Even though there is no direct evidence to prove the cellular and molecular changes induced by radiofrequency (RF) radiation itself, we cannot completely exclude the possibility of any biological effect of mobile phone frequency radiation. We established a carousel-type exposure chamber for 849 MHz or 1763 MHz of mobile phone RF radiation to expose RF to the heads of C57BL mice. In this chamber, animals were irradiated intermittently at 7.8 W/kg for a maximum of 12 months. During this period, the body weights of 3 groups-sham, 849 MHz RF, and 1763 MHz RF-did not show any differences between groups. The brain tissues were obtained from 3 groups at 6 months and 12 months to examine the differences in histology and cell proliferation between control and RF exposure groups, but we could not find any change upon RF radiation. Likewise, we could not find changes in the expression and distribution of NeuN and GFAP in hippocampus and cerebellum, or in cell death by TUNEL assay in RF exposure groups. From these data, we conclude that the chronic exposure to 849 MHz and 1763 MHz RF radiation at a 7.8 W/kg specific absorption rate (SAR) could not induce cellular alterations such as proliferation, death, and reactive gliosis.

(NE) Kleinlogel H, Dierks T, Koenig T, Lehmann H, Minder A, Berz R. Effects of weak mobile phone - electromagnetic fields (GSM, UMTS) on well-being and resting EEG. Bioelectromagnetics. 29(6):479-487, 2008a. (HU, BE, EE)

Modern mobile phones emit electromagnetic fields (EMFs) ranging from 900 to 2000 MHz which are suggested to have an influence on well-being, attention and neurological parameters in mobile phone users. To date most studies have investigated Global System for Mobile Communications (GSM)-EMF and only very few studies were concerned with Universal Mobile Telecommunications System (UMTS)-EMF. Consequently, we tested the effects of both types of EMF, 1950 MHz UMTS (SAR 0.1 and 1 W/kg) and pulsed 900 MHz GSM (1 W/kg), *on* well-being and vigilance-controlled resting electroencephalogram (eyes closed) in 15 healthy, right-handed subjects. A double-blind, randomised, crossover application of the test procedure was used. Neither the UMTS- nor the GSM-EMF produced any significant changes in the measured parameters compared to sham exposure. The results do not give any evidence for a deleterious effect of the EMF on normal healthy mobile phone users.

(NE) Kleinlogel H, Dierks T, Koenig T, Lehmann H, Minder A, Berz R. Effects of weak mobile phone - electromagnetic fields (GSM, UMTS) on event related potentials and cognitive functions. Bioelectromagnetics. 29(6):488-497, 2008b. (HU, EE, BE)

Modern mobile phones emit electromagnetic fields (EMF) ranging from 900 to 2000 MHz which are suggested to have an influence on well-being, attention and neurological parameters in mobile phone users. Until now most studies have investigated Global System for Mobile

Communications (GSM)-EMF and only very few studies have focused on Universal Mobile Telecommunications System (UMTS)-EMF. Therefore, we tested the effects of both types of unilaterally presented EMF, 1950 UMTS (0.1 and 1 W/kg) and pulsed 900 MHz GSM (1 W/kg), on visually evoked occipital P100, the P300 of a continuous performance test, auditory evoked central N100 and the P300 during an oddball task as well as on the respective behavioral parameters, reaction time and false reactions, in 15 healthy, right handed subjects. A double-blind, randomized, crossover application of the test procedure was used. Neither the UMTS- nor the GSM-EMF produced any significant changes in the measured parameters compared to sham exposure. The results do not give any evidence for a deleterious effect of the EMF on normal healthy mobile phone users.

(E) Köktürk S, Yardimoglu M, Celikozlu SD, Dolanbay EG, Cimbiz A. Effect of Lycopersicon esculentum extract on apoptosis in the rat cerebellum, following prenatal and postnatal exposure to an electromagnetic field. Exp Ther Med. 6(1):52-56, 2013. (AS, CE, DE, CC)

The expansion of mobile phone technology has raised concerns regarding the effect of 900-MHz electromagnetic field (EMF) exposure on the central nervous system. At present, the developing human brain is regularly exposed to mobile telephones, pre- and postnatally. Several studies have demonstrated the acute effects of EMF exposure during pre- or postnatal periods; however, the chronic effects of EMF exposure are less understood. Thus, the aim of the present study was to determine the chronic effects of EMF on the pre- and postnatal rat cerebellum. The control group was maintained in the same conditions as the experimental groups, without the exposure to EMF. In the EMF1 group, the rats were exposed to EMF during pre- and postnatal periods (until postnatal day 80). In the EMF2 group, the rats were also exposed to EMF pre- and postnatally; in addition, however, they were provided with a daily oral supplementation of *Lycopersicon esculentum* extract (~2 g/kg). The number of caspase-3-labeled Purkinje neurons and granule cells present in the rats in the control and experimental groups were then counted. The neurodegenerative changes were studied using cresyl violet staining, and these changes were evaluated. In comparison with the control animals, the EMF1 group demonstrated a significant increase in the number of caspase-3-labeled Purkinje neurons and granule cells present in the cerebellum (P<0.001). However, in comparison with the EMF1 group, the EMF2 group exhibited significantly fewer caspase-3-labeled Purkinje neurons and granule cells in the cerebellum. In the EMF1 group, the Purkinje neurons were revealed to have undergone dark neuron degenerative changes. However, the presence of dark Purkinje neurons was reduced in the EMF2 group, compared with the EMF1 group. The results indicated that apoptosis and neurodegeneration in rats exposed to EMF during pre- and postnatal periods may be reduced with *Lycopersicon esculentum* extract therapy.

(NE) Krause CM, Pesonen M, Haarala Björnberg C, Hämäläinen H. Effects of pulsed and continuous wave 902 MHz mobile phone exposure on brain oscillatory activity during cognitive processing. Bioelectromagnetics. 28(4):296-308, 2007. (HU, EE)

The aim of the current double-blind studies was to partially replicate the studies by Krause et al. [2000ab, 2004] and to further investigate the possible effects of electromagnetic fields (EMF) emitted by mobile phones (MP) on the event-related desynchronisation/synchronisation (ERD/ERS) EEG (electroencephalogram) responses during cognitive processing. Two groups, both consisting of 36 male participants, were recruited. One group performed an auditory memory task and the other performed a visual working memory task in six exposure conditions: SHAM (no EMF), CW (continuous wave EMF) and PM (pulse modulated EMF) during both left- and right-side exposure, while the EEG was recorded. In line with our previous studies, we observed that the exposure to EMF had modest effects on brain oscillatory responses in the alpha frequency range (approximately 8-12 Hz) and had no effects on the behavioural measures. The effects on the EEG were, however, varying, unsystematic and inconsistent with previous reports. We conclude that the effects of EMF on brain oscillatory responses may be subtle, variable and difficult to replicate for unknown reasons.

(E) Kumar RS, Sareesh NN, Nayak S, Mailankot M. Hypoactivity of Wistar rats exposed to mobile phone on elevated plus maze. Indian J Physiol Pharmacol. 53(3):283-286, 2009. (AS, BE)

No abstract available. From discussion section: "In conclusion, our preliminary results indicate mobile phone exposure induced behavioral changes in rats, expressed as deficit in open arm exploration on elevated plus-maze."

(E) Kumlin T, Iivonen H, Miettinen P, Juvonen A, van Groen T, Puranen L, Pitkäaho R, Juutilainen J, Tanila H. Mobile phone radiation and the developing brain: behavioral and morphological effects in juvenile rats. Radiat Res. 168(4):471-479, 2007. (AS, CE, ME, BE)

The increasing use of mobile phones by children and teenagers has raised concerns about their safety. Addressing such concerns is difficult, because no data are available on possible effects from long-term exposure to radiofrequency (RF) fields during the development of the nervous system. Possible morphological and functional changes were evaluated in the central nervous system of young male Wistar rats exposed to 900 MHz mobile phone signal for 2 h/day on 5 days/week. After 5 weeks of exposure at whole-body average specific energy absorption rates of 0.3 or 3.0 W/kg or sham exposure, six rats per group were examined histologically, and the remaining 18 rats per group were subjected to behavioral tests. No degenerative changes, dying neurons, or effects on the leakage of the blood-brain barrier were detected. No group differences were observed in the open-field test, plus maze test or acoustic startle response tests. In the water maze test, however, significantly improved learning ($P = 0.012$) and memory ($P = 0.01$) were detected in rats exposed to RF fields. The results do not indicate a serious threat to the developing brain from mobile phone radiation at intensities relevant to human exposure. However, the interesting finding of improved learning and memory warrants further studies.

(NE) Kwon MS, Jääskeläinen SK, Toivo T, Hämäläinen H. No effects of mobile phone electromagnetic field on auditory brainstem response. Bioelectromagnetics. 31(1):48-55, 2010a. (HU, EE)

The present study investigated the possible effects of the electromagnetic field (EMF) emitted by an ordinary GSM mobile phone (902.4 MHz pulsed at 217 Hz) on brainstem auditory processing. Auditory brainstem responses (ABR) were recorded in 17 healthy young adults, without a mobile phone at baseline, and then with a mobile phone on the ear under EMF-off and EMF-on conditions. The amplitudes, latencies, and interwave intervals of the main ABR components (waves I, III, V) were compared among the three conditions. ABR waveforms showed no significant differences due to exposure, suggesting that short-term exposure to mobile phone EMF did not affect the transmission of sensory stimuli from the cochlea up to the midbrain along the auditory nerve and brainstem auditory pathways.

(NE) Kwon MS, Huotilainen M, Shestakova A, Kujala T, Näätänen R, Hämäläinen H. No effects of mobile phone use on cortical auditory change-detection in children: an ERP study. Bioelectromagnetics. 31(3):191-199, 2010b. (HU, EE)

We investigated the effect of mobile phone use on the auditory sensory memory in children. Auditory event-related potentials (ERPs), P1, N2, mismatch negativity (MMN), and P3a, were recorded from 17 children, aged 11-12 years, in the recently developed multi-feature paradigm. This paradigm allows one to determine the neural change-detection profile consisting of several different types of acoustic changes. During the recording, an ordinary GSM (Global System for Mobile Communications) mobile phone emitting 902 MHz (pulsed at 217 Hz) electromagnetic field (EMF) was placed on the ear, over the left or right temporal area (SAR(1g) = 1.14 W/kg, SAR(10g) = 0.82 W/kg, peak value = 1.21 W/kg). The EMF was either on or off in a single-blind manner. We found that a short exposure (two 6 min blocks for each side) to mobile phone EMF has no statistically significant effects on the neural change-detection profile measured with the MMN. Furthermore, the multi-feature paradigm was shown to be well suited for studies of perception accuracy and sensory memory in children. However, it should be noted that the present study only had sufficient statistical power to detect a large effect size.

(NE) Kwon MS, Vorobyev V, Kännälä S, Laine M, Rinne JO, Toivonen T, Johansson J, Teräs M, Joutsa J, Tuominen L, Lindholm H, Alanko T, Hämäläinen H. No effects of short-term GSM mobile phone radiation on cerebral blood flow measured using positron emission tomography. Bioelectromagnetics. 33(3):247-256, 2012. (HU, PE)

The present study investigated the effects of 902.4 MHz global system for mobile communications (GSM) mobile phone radiation on cerebral blood flow using positron emission tomography (PET) with the (15) O-water tracer. Fifteen young, healthy, right-handed male subjects were exposed to phone radiation from three different locations (left ear, right ear, forehead) and to sham exposure to test for possible exposure effects on brain regions close to the exposure source. Whole-brain $[^{15}O]H_2O$-PET images were acquired 12 times, 3 for each condition, in a counterbalanced order. Subjects were exposed for 5 min in each scan while performing a simple visual vigilance task. Temperature was also measured in the head region (forehead, eyes, cheeks, ear canals) during exposure. The exposure induced a slight temperature rise in the ear canals but did not affect brain hemodynamics and task performance. The results provided no evidence for acute effects of short-term mobile phone radiation on cerebral blood flow.

(E) Kwon MS, Vorobyev V, Kännälä S, Laine M, Rinne JO, Toivonen T, Johansson J, Teräs M, Lindholm H, Alanko T, Hämäläinen H. GSM mobile phone radiation suppresses brain glucose metabolism. J Cereb Blood Flow Metab. 31(12):2293-2301, 2011. (HU, PE)

We investigated the effects of mobile phone radiation on cerebral glucose metabolism using high-resolution positron emission tomography (PET) with the (18)F-deoxyglucose (FDG) tracer.

A long half-life (109 minutes) of the (18)F isotope allowed a long, natural exposure condition outside the PET scanner. Thirteen young right-handed male subjects were exposed to a pulse-modulated 902.4 MHz Global System for Mobile Communications signal for 33 minutes, while performing a simple visual vigilance task. Temperature was also measured in the head region (forehead, eyes, cheeks, ear canals) during exposure. (18)F-deoxyglucose PET images acquired after the exposure showed that relative cerebral metabolic rate of glucose was significantly reduced in the temporoparietal junction and anterior temporal lobe of the right hemisphere ipsilateral to the exposure. Temperature rise was also observed on the exposed side of the head, but the magnitude was very small. The exposure did not affect task performance (reaction time, error rate). Our results show that short-term mobile phone exposure can locally suppress brain energy metabolism in humans.

(NE) Kwon MS, Kujala T, Huotilainen M, Shestakova A, Näätänen R, Hämäläinen H. Preattentive auditory information processing under exposure to the 902 MHz GSM mobile phone electromagnetic field: a mismatch negativity (MMN) study. Bioelectromagnetics. 30(3):241-248, 2009. (HU, EE)

Previous studies on the effects of the mobile phone electromagnetic field (EMF) on various event-related potential (ERP) components have yielded inconsistent and even contradictory results, and often failed in replication. The mismatch negativity (MMN) is an auditory ERP component elicited by infrequent (deviant) stimuli differing in some physical features from the repetitive frequent (standard) stimuli in a sound sequence. The MMN provides a sensitive measure for cortical auditory stimulus feature discrimination, regardless of attention and other contaminating factors. In this study, MMN responses to duration, intensity, frequency, and gap changes were recorded in healthy young adults (n = 17), using a multifeature paradigm including several types of auditory change in the same stimulus sequence, while a GSM mobile phone was placed on either ear with the EMF (902 MHz pulsed at 217 Hz; SAR(1g) = 1.14 W/kg, SAR(10g) = 0.82 W/kg, peak value = 1.21 W/kg, measured with an SAM phantom) on or off. An MMN was elicited by all deviant types, while its amplitude and latency showed no significant differences due to EMF exposure for any deviant types. In the present study, we found no conclusive evidence that acute exposure to GSM mobile phone EMF affects cortical auditory change detection processing reflected by the MMN.

(E) Lee KS, Choi JS, Hong SY, Son TH, Yu K. Mobile phone electromagnetic radiation activates MAPK signaling and regulates viability in Drosophila. Bioelectromagnetics. 29(5):371-379, 2008. (AS, CC)

Mobile phones are widely used in the modern world. However, biological effects of electromagnetic radiation produced by mobile phones are largely unknown. In this report, we show biological effects of the mobile phone 835 MHz electromagnetic field (EMF) in the Drosophila model system. When flies were exposed to the specific absorption rate (SAR) 1.6 W/kg, which is the proposed exposure limit by the American National Standards Institute (ANSI), more than 90% of the flies were viable even after the 30 h exposure. However, in the SAR 4.0 W/kg strong EMF exposure, viability dropped from the 12 h exposure. These EMF exposures triggered stress response and increased the production of reactive oxygen species. The EMF exposures also activated extracellular signal regulated kinase (ERK) and c-Jun N-terminal kinase (JNK) signaling, but not p38 kinase signaling. Interestingly, SAR 1.6 W/kg activated mainly ERK signaling and expression of an anti-apoptotic gene, whereas SAR 4.0 W/kg strongly activated JNK signaling and expression of apoptotic genes. In addition, SAR 4.0 W/kg amplified the number of apoptotic cells in the fly brain. These findings demonstrate that the exposure limit on electromagnetic radiation proposed by ANSI triggered ERK-survival signaling but the strong electromagnetic radiation activated JNK-apoptotic signaling in Drosophila.

(E) Leung S, Croft RJ, McKenzie RJ, Iskra S, Silber B, Cooper NR, O'Neill B, Cropley V, Diaz-Trujillo A, Hamblin D, Simpson D. Effects of 2G and 3G mobile phones on performance and electrophysiology in adolescents, young adults and older adults. Clin Neurophysiol. 122(11):2203-2216, 2011. (HU, AD, BE, EE)

OBJECTIVE: This study examined sensory and cognitive processing in adolescents, young adults and older adults, when exposed to 2nd (2G) and 3rd (3G) generation mobile phone signals. **METHODS:** Tests employed were the auditory 3-stimulus oddball and the N-back. Forty-one 13-15 year olds, forty-two 19-40 year olds and twenty 55-70 year olds were tested using a double-blind cross-over design, where each participant received Sham, 2G and 3G exposures, separated by at least 4 days. **RESULTS:** 3-Stimulus oddball task: Behavioural: accuracy and reaction time of responses to targets were not affected by exposure. Electrophysiological: augmented N1 was found in the 2G condition (independent of age group). N-back task: Behavioural: the combined groups performed less accurately during the 3G exposure (compared to Sham), with post hoc tests finding this effect separately in the adolescents only. Electrophysiological: delayed ERD/ERS responses of the alpha power were found in both 3G and 2G conditions (compared to Sham; independent of age group). **CONCLUSION:** Employing tasks tailored to each individual's ability level, this study provides support for an effect of acute 2G and 3G exposure on human cognitive function. **SIGNIFICANCE:** The subtlety of mobile phone effect on cognition in our study suggests that it is important to account for individual differences in future mobile phone research.

(NE) Lipping T, Rorarius M, Jäntti V, Annala K, Mennander A, Ferenets R, Toivonen T, Toivo T, Värri A, Korpinen L. Using the nonlinear control of anaesthesia-induced hypersensitivity of EEG at burst suppression level to test the effects of radiofrequency radiation on brain function. Nonlinear Biomed Phys. 3(1):5, 2009. (AS, IA, EE)

BACKGROUND: In this study, investigating the effects of mobile phone radiation on test animals, eleven pigs were anaesthetised to the level where burst-suppression pattern appears in the electroencephalogram (EEG). At this level of anaesthesia both human subjects and animals show high sensitivity to external stimuli which produce EEG bursts during suppression. The burst-suppression phenomenon represents a nonlinear control system, where low-amplitude EEG abruptly switches to very high amplitude bursts. This switching can be triggered by very minor stimuli and the phenomenon has been described as hypersensitivity. To test if also radio frequency (RF) stimulation can trigger this nonlinear control, the animals were exposed to pulse modulated signal of a GSM mobile phone at 890 MHz. In the first phase of the experiment electromagnetic field (EMF) stimulation was randomly switched on and off and the relation between EEG bursts and EMF stimulation onsets and endpoints were studied. In the second phase a continuous RF stimulation at 31 W/kg was applied for 10 minutes. The ECG, the EEG, and the subcutaneous temperature were recorded. **RESULTS:** No correlation between the exposure and the EEG burst occurrences was observed in phase I measurements. No significant changes were observed in the EEG activity of the pigs during phase II measurements although several EEG signal analysis methods were applied. The temperature measured subcutaneously from the pigs' head increased by 1.6 degrees C and the heart rate by 14.2 bpm on the average during the 10 min exposure periods. **CONCLUSION:** The hypothesis that RF radiation would produce sensory stimulation of somatosensory, auditory or visual system or directly affect the brain so as to produce EEG bursts during suppression was not confirmed.

(E) Liu ML, Wen JQ, Fan YB. Potential protection of green tea polyphenols against 1800 MHz electromagnetic radiation-induced injury on rat cortical neurons. Neurotox Res. 20(3):270-276, 2011. (CS, IA, CC, OX)

Radiofrequency electromagnetic fields (EMF) are harmful to public health, but the certain anti-irradiation mechanism is not clear yet. The present study was performed to investigate the possible protective effects of green tea polyphenols against electromagnetic radiation-induced injury in the cultured rat cortical neurons. In this study, green tea polyphenols were used in the cultured cortical neurons exposed to 1800 MHz EMFs by the mobile phone. We found that the mobile phone irradiation for 24 h induced marked neuronal cell death in the MTT (3-(4,5-dimethylthiazole-2-yl)-2,5-diphenyl-tetrazolium bromide) and TUNEL (TdT mediated biotin-dUTP nicked-end labeling) assay, and protective effects of green tea polyphenols on the injured cortical neurons were demonstrated by testing the content of Bcl-2 Assaciated X protein (Bax) in the immunoprecipitation assay and Western blot assay. In our study results, the mobile phone irradiation-induced increases in the content of active Bax were inhibited significantly by green tea polyphenols, while the contents of total Bax had no marked changes after the treatment of green tea polyphenols. Our results suggested a neuroprotective effect of green tea polyphenols against the mobile phone irradiation-induced injury on the cultured rat cortical neurons.

(E) Liu YX, Tai JL, Li GQ, Zhang ZW, Xue JH, Liu HS, Zhu H, Cheng JD, Liu YL, Li AM, Zhang Y. Exposure to 1950-MHz TD-SCDMA Electromagnetic Fields Affects the Apoptosis of Astrocytes via Caspase-3-Dependent Pathway. PLoS One. 7(8):e42332, 2012. (CS, CC)

The usage of mobile phone increases globally. However, there is still a paucity of data about the impact of electromagnetic fields (EMF) on human health. This study investigated whether EMF radiation would alter the biology of glial cells and act as a tumor-promoting agent. We exposed rat astrocytes and C6 glioma cells to 1950-MHz TD-SCDMA for 12, 24 and 48 h respectively, and found that EMF exposure had differential effects on rat astroctyes and C6 glioma cells. A 48 h of exposure damaged the mitochondria and induced significant apoptosis of astrocytes. Moreover, caspase-3, a hallmark of apoptosis, was highlighted in astrocytes after 48 h of EMF exposure, accompanied by a significantly increased expression of bax and reduced level of bcl-2. The tumorigenicity assays demonstrated that astrocytes did not form tumors in both control and exposure groups. In contrast, the unexposed and exposed C6 glioma cells show no significant differences in both biological feature and tumor formation ability. Therefore, our results implied that exposure to the EMF of 1950-MHz TD-SCDMA may not promote the tumor formation, but continuous exposure damaged the mitochondria of astrocytes and induce apoptosis through a caspase-3-dependent pathway with the involvement of bax and bcl-2.

(E) López-Martín E, Bregains J, Relova-Quinteiro JL, Cadarso-Suárez C, Jorge-Barreiro FJ, Ares-Pena FJ. The action of pulse-modulated GSM radiation increases regional changes in brain activity and c-Fos expression in cortical and subcortical areas in a rat model of picrotoxin-induced seizure proneness. J Neurosci Res. 87(6):1484-1499, 2009. (AS, CC, WS)

The action of the pulse-modulated GSM radiofrequency of mobile phones has been suggested as a physical phenomenon that might have biological effects on the mammalian central nervous system. In the present study, GSM-exposed picrotoxin-pretreated rats showed differences in clinical and EEG signs, and in c-Fos expression in the brain, with respect to picrotoxin-treated rats exposed to an equivalent dose of unmodulated radiation. Neither radiation treatment caused tissue heating, so thermal effects can be ruled out. The most marked effects of GSM radiation on c-Fos expression in picrotoxin-treated rats were observed in limbic structures, olfactory cortex areas and subcortical areas, the dentate gyrus, and the central lateral nucleus of the thalamic intralaminar nucleus group. Nonpicrotoxin-treated animals exposed to unmodulated radiation showed the highest levels of neuronal c-Fos expression in cortical areas. These results suggest a specific effect of the pulse modulation of GSM radiation on brain activity of a picrotoxin-induced seizure-proneness rat model and indicate that this mobile-phone-type radiation might induce regional changes in previous preexcitability conditions of neuronal activation.

(E) Loughran SP, McKenzie RJ, Jackson ML, Howard ME, Croft RJ. Individual differences in the effects of mobile phone exposure on human sleep: rethinking the problem. Bioelectromagnetics. 33(1):86-93, 2012. (HU, EE, SL)

Mobile phone exposure-related effects on the human electroencephalogram (EEG) have been shown during both waking and sleep states, albeit with slight differences in the frequency affected. This discrepancy, combined with studies that failed to find effects, has led many to conclude that no consistent effects exist. We hypothesised that these differences might partly be due to individual variability in response, and that mobile phone emissions may in fact have large but differential effects on human brain activity. Twenty volunteers from our previous study underwent an adaptation night followed by two experimental nights in which they were randomly exposed to two conditions (Active and Sham), followed by a full-night sleep episode. The EEG spectral power was increased in the sleep spindle frequency range in the first 30 min of non-rapid eye movement (non-REM) sleep following Active exposure. This increase was more prominent in the participants that showed an increase in the original study. These results confirm previous findings of mobile phone-like emissions affecting the EEG during non-REM sleep. Importantly, this low-level effect was also shown to be sensitive to individual variability. Furthermore, this indicates that previous negative results are not strong evidence for a lack of an effect and, given the far-reaching implications of mobile phone research, we may need to rethink the interpretation of results and the manner in which research is conducted in this field.

(NE) Loughran SP, Benz DC, Schmid MR, Murbach M, Kuster N, Achermann P. No increased sensitivity in brain activity of adolescents exposed to mobile phone-like emissions. Clin Neurophysiol. 124(7):1303-1308, 2013. (HU, BE, EE, AD)

OBJECTIVE: To examine the potential sensitivity of adolescents to radiofrequency electromagnetic field (RF EMF) exposures, such as those emitted by mobile phones. **METHODS:** In a double-blind, randomized, crossover design, 22 adolescents aged 11-13years (12 males) underwent three experimental sessions in which they were exposed to mobile phone-like RF EMF signals at two different intensities, and a sham session. During exposure cognitive tasks were performed and waking EEG was recorded at three time-points subsequent to exposure (0, 30 and 60min). **RESULTS:** No clear significant effects of RF EMF exposure were found on the waking EEG or cognitive performance. **CONCLUSIONS:** Overall, the current study was unable to demonstrate exposure-related effects previously observed on the waking EEG in adults, and also provides further support for a lack of an influence of mobile phone-like exposure on cognitive performance. **SIGNIFICANCE:** Adolescents do not appear to be more sensitive than adults to mobile phone RF EMF emissions.

(E) Lowden A, Akerstedt T, Ingre M, Wiholm C, Hillert L, Kuster N, Nilsson JP, Arnetz B. Sleep after mobile phone exposure in subjects with mobile phone-related symptoms. Bioelectromagnetics. 32(1):4-14, 2011. (HU, EE, SL)

Several studies show increases in activity for certain frequency bands (10-14 Hz) and visually scored parameters during sleep after exposure to radiofrequency electromagnetic fields. A shortened REM latency has also been reported. We investigated the effects of a double-blind radiofrequency exposure (884 MHz, GSM signaling standard including non-DTX and DTX mode, time-averaged 10 g psSAR of 1.4 W/kg) on self-evaluated sleepiness and objective EEG measures during sleep. Forty-eight subjects (mean age 28 years) underwent 3 h of controlled exposure (7:30-10:30 PM; active or sham) prior to sleep, followed by a full-night polysomnographic recording in a sleep laboratory. The results demonstrated that following exposure, time in Stages 3 and 4 sleep (SWS, slow-wave sleep) decreased by 9.5 min (12%) out of a total of 78.6 min, and time in Stage 2 sleep increased by 8.3 min (4%) out of a total of 196.3 min compared to sham. The latency to Stage 3 sleep was also prolonged by 4.8 min after exposure. Power density analysis indicated an enhanced activation in the frequency ranges 0.5-1.5 and 5.75-10.5 Hz during the first 30 min of Stage 2 sleep, with 7.5-11.75 Hz being elevated within the first hour of Stage 2 sleep, and bands 4.75-8.25 Hz elevated during the second hour of Stage 2 sleep. No pronounced power changes were observed in SWS or for the third hour of scored Stage 2 sleep. No differences were found between controls and subjects with prior complaints of mobile phone-related symptoms. The results confirm previous findings that RF exposure increased the EEG alpha range in the sleep EEG, and indicated moderate impairment of SWS. Furthermore, reported differences in sensitivity to mobile phone use were not reflected in sleep parameters.

(E) Lu Y, Xu S, He M, Chen C, Zhang L, Liu C, Chu F, Yu Z, Zhou Z, Zhong M. Glucose administration attenuates spatial memory deficits induced by chronic low-power-density microwave exposure. Physiol Behav. 106(5):631-637, 2012. (AS, CE, BE)

Extensive evidence indicates that glucose administration attenuates memory deficits in rodents and humans, and cognitive impairment has been associated with reduced glucose metabolism and uptake in certain brain regions including the hippocampus. In the present study, we investigated whether glucose treatment attenuated memory deficits caused by chronic low-power-density microwave (MW) exposure, and the effect of MW exposure on hippocampal glucose uptake. We exposed Wistar rats to 2.45 GHz pulsed MW irradiation at a power density of 1 mW/cm(2) for 3 h/day, for up to 30 days. MW exposure induced spatial learning and memory impairments in rats. Hippocampal glucose uptake was also reduced by MW exposure in the absence or presence of insulin, but the levels of blood glucose and insulin were not affected. However, these spatial memory deficits were reversed by systemic glucose treatment. Our results indicate that glucose administration attenuates the spatial memory deficits induced by chronic low-power-density MW exposure, and reduced hippocampal glucose uptake may be associated with cognitive impairment caused by MW exposure.

(E) Luria R, Eliyahu I, Hareuveny R, Margaliot M, Meiran N. Cognitive effects of radiation emitted by cellular phones: the influence of exposure side and time. Bioelectromagnetics. 30(3):198-204, 2009. (See also Hareuveny et al., 2011) (HU, BE)

This study examined the time dependence effects of exposure to radiofrequency radiation (RFR) emitted by standard GSM cellular phones on the cognitive functions of humans. A total of 48 healthy right-handed male subjects performed a spatial working memory task (that required either a left-hand or a right-hand response) while being exposed to one of two GSM phones placed at both sides of the head. The subjects were randomly divided into three groups. Each group was exposed to one of three exposure conditions: left-side of the head, right-side, or sham-exposure. The experiment consisted of 12 blocks of trials. Response times (RTs) and accuracy of the responses were recorded. It was found that the average RT of the right-hand responses under left-side exposure condition was significantly longer than those of the right-side and sham-exposure groups averaged together during the first two time blocks. These results confirmed the existence of an effect of exposure on RT, as well as the fact that exposure duration (together with the responding hand and the side of exposure) may play an important role in producing detectable RFR effects on performance. Differences in these parameters might be the reason for the failure of certain studies to detect or replicate RFR effects.

(E) Lustenberger C, Murbach M, Durr R, Schmid MR, Kuster N, Achermann P, Huber R. Stimulation of the brain with radiofrequency electromagnetic field pulses affects sleep-dependent performance improvement. Brain Stimul 6(5):805-811, 2013. (HU, BE, EE, SL)

Background: Sleep-dependent performance improvements seem to be closely related to sleep spindles (12–15 Hz) and sleep slow-wave activity (SWA, 0.75–4.5 Hz). Pulse-modulated radiofrequency electromagnetic fields (RF EMF, carrier frequency 900 MHz) are capable to modulate these electroencephalographic (EEG) characteristics of sleep. Objective: The aim of our study was to explore possible mechanisms how RF EMF affects cortical activity during sleep and to test whether such effects on cortical activity during sleep interact with sleep-dependent performance changes. Methods: Sixteen male subjects underwent 2 experimental nights, one of them with all-night 0.25–0.8 Hz pulsed RF EMF exposure. All-night EEG was recorded. To investigate RF EMF induced changes in overnight performance improvement, subjects were trained for both nights on a motor task in the evening and the morning. Results: We obtained good sleep quality in all subjects under both conditions (mean sleep efficiency > 90%). After pulsed RF EMF we found increased SWA during exposure to pulse-modulated RF EMF compared to sham exposure ($P < 0.05$) toward the end of the sleep period. Spindle activity was not affected. Moreover, subjects showed an increased RF EMF burst-related response in the SWA range, indicated by an increase in event-related EEG spectral power and phase changes in the SWA range. Notably, during exposure, sleep-dependent performance improvement in the motor sequence task was reduced compared to the sham condition (−20.1%, *P* = 0.03). Conclusion: The changes in the time course of SWA during the exposure night may reflect an interaction of RF EMF with the renormalization of cortical excitability during sleep, with a negative impact on sleep-dependent performance improvement.

(E) Lv B, Chen Z, Wu T, Shao Q, Yan D, Ma L, Lu K, Xie Y. The alteration of spontaneous low frequency oscillations caused by acute electromagnetic fields **exposure. Clin Neurophysiol. 2013 Sep 4. pii: S1388-2457(13)00976-0. doi: 10.1016/j.clinph.2013.07.018. [Epub ahead of print] (HU, EE, PE)**

OBJECTIVE: The motivation of this study is to evaluate the possible alteration of regional resting state brain activity induced by the acute radiofrequency electromagnetic field (RF-EMF) exposure (30min) of Long Term Evolution (LTE) signal. METHODS: We designed a controllable near-field LTE RF-EMF exposure environment. Eighteen subjects participated in a double-blind, crossover, randomized and counterbalanced experiment including two sessions (real and sham exposure). The radiation source was close to the right ear. Then the resting state fMRI signals of human brain were collected before and after the exposure in both sessions. We measured the amplitude of low frequency fluctuation (ALFF) and fractional ALFF (fALFF) to characterize the spontaneous brain activity. RESULTS: We found the decreased ALFF value around in left superior temporal gyrus, left middle temporal gyrus, right superior temporal gyrus, right medial frontal gyrus and right paracentral lobule after the real exposure. And the decreased fALFF value was also detected in right medial frontal gyrus and right paracentral lobule. CONCLUSIONS: The study provided the evidences that 30min LTE RF-EMF exposure modulated the spontaneous low frequency fluctuations in some brain regions. SIGNIFICANCE: With resting state fMRI, we found the alteration of spontaneous low frequency fluctuations induced by the acute LTE RF-EMF exposure.

(E) Maaroufi K, Had-Aissouni L, Melon C, Sakly M, Abdelmelek H, Poucet B, Save E. Spatial learning, monoamines and oxidative stress in rats exposed to 900MHz electromagnetic field in combination with iron overload. Behav Brain Res. 2013 Oct 18. pii: S0166-4328(13)00624-4. doi: 10.1016/j.bbr.2013.10.016. [Epub ahead of print] (AS, CE, BE, CH)

The increasing use of mobile phone technology over the last decade raises concerns about the impact of high frequency electromagnetic fields (EMF) on health. More recently, a link between EMF, iron overload in the brain and neurodegenerative disorders including Parkinson's and Alzheimer's diseases has been suggested. Co-exposure to EMF and brain iron overload may have a greater impact on brain tissues and cognitive processes than each treatment by itself. To examine this hypothesis, Long-Evans rats submitted to 900MHz exposure or combined 900MHz EMF and iron overload treatments were tested in various spatial learning tasks (navigation task in the Morris water maze, working memory task in the radial-arm maze, and object exploration task involving spatial and non spatial processing). Biogenic monoamines and metabolites (dopamine, serotonin) and oxidative stress were measured. Rats exposed to EMF were impaired in the object exploration task but not in the navigation and working memory tasks. They also showed alterations of monoamine content in several brain areas but mainly in the hippocampus. Rats that received combined treatment did not show greater behavioral and neurochemical deficits than EMF-exposed rats. None of the two treatments produced global oxidative stress. These results show that there is an impact of EMF on the brain and cognitive processes but this impact is revealed only in a task exploiting spontaneous exploratory activity. In contrast, there are no synergistic effects between EMF and a high content of iron in the brain.

(E) Maganioti AE, Hountala CD, Papageorgiou CC, Kyprianou MA, Rabavilas AD, Capsalis CN. Principal component analysis of the P600 waveform: RF and gender effects. Neurosci Lett. 478(1):19-23, 2010. (HU, EE)

The aim of the present study was to examine the patterns of activation of the P600 waveform of the event-related potentials (ERP), applying principal component analysis (PCA) and repeated measures ANOVA, and whether these patterns are RF and gender dependent. The ERPs of thirty-nine healthy subjects (20 male and 19 female) were recorded during an auditory memory task in the presence and absence of RF, similar to that emitted by mobile phones. Both PCA and ANOVA produced congruent results, showing that activation of the P600 component occurs early and more intensely in the region of the posterior electrodes and in a less intense manner in the central electrodes. Conversely, the activation at the anterior electrodes arises later with a considerably reduced intensity. In the absence of RF female subjects exhibited significantly lower amplitudes at anterior electrodes and earlier latencies at central electrodes than male subjects. These differences disappear in the presence of RF. Consequently, the P600 component follows distinct patterns of activation in the anterior, central and posterior brain areas and gender differences are observed simultaneously at several electrodes within these areas. Finally, the gender-related functional architecture with regard the P600 component appears to be RF sensitive. In conclusion, the application of the PCA procedure provides an adequate model of the spatially distributed event-related dynamics that correspond to the P600 waveform.

(E) Mandalà M, Colletti V, Sacchetto L, Manganotti P, Ramat S, Marcocci A, Colletti L. Effect of Bluetooth headset and mobile phone electromagnetic fields on the human auditory nerve. Laryngoscope. 2013 Apr 25. doi: 10.1002/lary.24103. [Epub ahead of print] (HU, EE)

OBJECTIVES/HYPOTHESIS: The possibility that long-term mobile phone use increases the incidence of astrocytoma, glioma and acoustic neuroma has been investigated in several studies. Recently, our group showed that direct exposure (in a surgical setting) to cell phone electromagnetic fields (EMFs) induces deterioration of auditory evoked cochlear nerve compound action potential (CNAP) in humans. To verify whether the use of Bluetooth devices reduces these effects, we conducted the present study with the same experimental protocol. STUDY DESIGN: Randomized trial. METHODS: Twelve patients underwent retrosigmoid vestibular neurectomy to treat definite unilateral Ménière's disease while being monitored with acoustically evoked CNAPs to assess direct mobile phone exposure or alternatively the EMF effects of Bluetooth headsets. RESULTS: We found no short-term effects of Bluetooth EMFs on the auditory nervous structures, whereas direct mobile phone EMF exposure confirmed a significant decrease in CNAPs amplitude and an increase in latency in all subjects. CONCLUSIONS: The outcomes of the present study show that, contrary to the finding that the latency and amplitude of CNAPs are very sensitive to EMFs produced by the tested mobile phone, the EMFs produced by a common Bluetooth device do not induce any significant change in cochlear nerve activity. The conditions of exposure, therefore, differ from those of everyday life, in which various biological tissues may reduce the EMF affecting the cochlear nerve. Nevertheless, these novel findings may have important safety implications.

(E) Masuda H, Hirata A, Kawai H, Wake K, Watanabe S, Arima T, Poulletier de Gannes F, Lagroye I, Veyret B. Local exposure of the rat cortex to radiofrequency electromagnetic fields increases local cerebral blood flow along with temperature. J Appl Physiol. 110(1):142-148, 2011. (AS, PE)

Few studies have shown that local exposure to radiofrequency electromagnetic fields (RF) induces intensity-dependent physiological changes, especially in the brain. The aim of the present study was to detect reproducible responses to local RF exposure in the parietal cortex of anesthetized rats and to determine their dependence on RF intensity. The target cortex tissue was locally exposed to 2-GHz RF using a figure-eight loop antenna within a range of averaged specific absorption rates (10.5, 40.3, 130, and 263 W/kg averaged over 4.04 mg*)* in the target area. Local cerebral blood flow (CBF) and temperatures in three regions (target area, rectum, and calf hypodermis) were measured using optical fiber blood flow meters and thermometers during RF exposure. All parameters except for the calf hypodermis temperature increased significantly in exposed animals compared with sham-exposed ones during 18-min exposures. Dependence of parameter values on exposure intensity was analyzed using linear regression models. The elevation of local CBF was correlated with temperature rise in both target and rectum at the end of RF exposure. However, the local CBF elevation seemed to be elevated by the rise in target temperature, but not by that of the rectal temperature, in the early part of RF exposure or at low-intensity RF exposure. These findings suggest that local RF exposure of the rat cortex drives a regulation of CBF accompanied by a local temperature rise, and our findings may be helpful for discussing physiological changes in the local cortex region, which is locally exposed to RF.

(E) Maskey D, Kim M, Aryal B, Pradhan J, Choi IY, Park KS, Son T, Hong SY, Kim SB, Kim HG, Kim MJ. Effect of 835 MHz radiofrequency radiation exposure on calcium binding proteins in the hippocampus of the mouse brain. Brain Res. 1313:232-241, 2010a. (AS, CE, ME, CH)

Worldwide expansion of mobile phones and electromagnetic field (EMF) exposure has raised question of their possible biological effects on the brain and nervous system. Radiofrequency (RF) radiation might alter intracellular signaling pathways through changes in calcium (Ca(2+)) permeability across cell membranes. Changes in the expression of calcium binding proteins (CaBP) like calbindin D28-k (CB) and calretinin (CR) could indicate impaired Ca(2+)homeostasis due to EMF exposure. CB and CR expression were measured with immunohistochemistry in the hippocampus of mice after EMF exposure at 835 MHz for different exposure times and absorption rates, 1 h/day for 5 days at a specific absorption rate (SAR)=1.6 W/kg, 1 h/day for 5 days at SAR=4.0 W/kg, 5 h/day for 1 day at SAR=1.6 W/kg, 5 h/day for 1 day at SAR=4.0 W/kg, daily exposure for 1 month at SAR=1.6 W/kg. Body weights did not change significantly. CB immunoreactivity (IR) displayed moderate staining of cells in the cornu ammonis (CA) areas and prominently stained granule cells. CR IR revealed prominently stained pyramidal cells with dendrites running perpendicularly in the CA area. Exposure for 1 month produced almost complete loss of pyramidal cells in the CA1 area. CaBP differences could cause changes in cellular Ca(2+)levels, which could have deleterious effect on normal hippocampal functions concerned with neuronal connectivity and integration.

(E) **Maskey D, Pradhan J, Aryal B, Lee CM, Choi IY, Park KS, Kim SB, Kim HG, Kim MJ. Chronic 835-MHz radiofrequency exposure to mice hippocampus alters the distribution of calbindin and GFAP immunoreactivity. Brain Res. 1346:237-246, 2010b.** **(AS, CE, ME, CH)**

Exponential interindividual handling in wireless communication system has raised possible doubts in the biological aspects of radiofrequency (RF) exposure on human brain owing to its close proximity to the mobile phone. In the nervous system, calcium (Ca(2+)) plays a critical role in releasing neurotransmitters, generating action potential and membrane integrity. Alterations in intracellular Ca(2+) concentration trigger aberrant synaptic action or cause neuronal apoptosis, which may exert an influence on the cellular pathology for learning and memory in the hippocampus. Calcium binding proteins like calbindin D28-K (CB) is responsible for the maintaining and controlling Ca(2+) homeostasis. Therefore, in the present study, we investigated the effect of RF exposure on rat hippocampus at 835 MHz with low energy (specific absorption rate: SAR=1.6 W/kg) for 3 months by using both CB and glial fibrillary acidic protein (GFAP) specific antibodies by immunohistochemical method. Decrease in CB immunoreactivity (IR) was noted in exposed (E1.6) group with loss of interneurons and pyramidal cells in CA1 area and loss of granule cells. Also, an overall increase in GFAP IR was observed in the hippocampus of E1.6. By TUNEL assay, apoptotic cells were detected in the CA1, CA3 areas and dentate gyrus of hippocampus, which reflects that chronic RF exposure may affect the cell viability. In addition, the increase of GFAP IR due to RF exposure could be well suited with the feature of reactive astrocytosis, which is an abnormal increase in the number of astrocytes due to the loss of nearby neurons. Chronic RF exposure to the rat brain suggested that the decrease of CB IR accompanying apoptosis and increase of GFAP IR might be morphological parameters in the hippocampus damages.

(E) **Maskey D, Kim HJ, Kim HG, Kim MJ. Calcium-binding proteins and GFAP immunoreactivity alterations in murine hippocampus after 1 month of exposure to 835 MHz radiofrequency at SAR values of 1.6 and 4.0 W/kg. Neurosci Lett. 506(2):292-296, 2012.** **(AS, CE, ME, CH)**

Widespread use of wireless mobile communication has raised concerns of adverse effect to the brain owing to the proximity during use due to the electromagnetic field emitted by mobile phones. Changes in calcium ion concentrations via binding proteins can disturb calcium homeostasis; however, the correlation between calcium-binding protein (CaBP) immunoreactivity (IR) and glial cells has not been determined with different SAR values. Different SAR values [1.6 (E1.6 group) and 4.0 (E4 group) W/kg] were applied to determine the distribution of calbindin D28-k (CB), calretinin (CR), and glial fibrillary acidic protein (GFAP) IR in murine hippocampus. Compared with sham control group, decreased CB and CR IRs, loss of CB and CR immunoreactive cells and increased GFAP IR exhibiting hypertrophic cytoplasmic processes were noted in both experimental groups. E4 group showed a prominent decrement in CB and CR IR than the E1.6 group due to down-regulation of CaBP proteins and neuronal loss. GFAP IR was more prominent in the E4 group than the E1.6 group. Decrement in the CaBPs can affect the calcium-buffering capacity leading to cell death, while increased GFAP IR and changes in astrocyte morphology, may mediate brain injury due to radiofrequency exposure.

(E) Maskey D, Kim MJ. Immunohistochemical Localization of Brain-derived Neurotrophic Factor and Glial Cell Line-derived Neurotrophic Factor in the Superior Olivary Complex of Mice after Radiofrequency Exposure. Neuroscience Letters. Available online February 16, 2014. (AS, CE, CH)

Raising health concerns about the biological effects from radiofrequency exposure, even with conflicting results, has prompted calls for formulation of a guideline of the biological safety level. Given the close proximity between a mobile phone and the ear, it has been suggested that the central auditory system may be detrimentally influenced by radiofrequency exposure. In the auditory system, neurotrophins are important in the regulation of neuron survival, especially mammalian cochlear neurons. Neurotrophic factors like brain-derived neurotrophic factor (BDNF) and glial-derived neurotrophic factor (GDNF) present in the auditory system are responsible for the maintenance of auditory neurons. BDNF and GDNF may protect against acoustic trauma and prevent from hearing defect. The present study applied radiofrequency at a specific absorption rate (SAR) of 1.6 W/kg (E1.6) or 0 W/kg group to determine the distribution of BDNF and GDNF in the nuclei of superior olivary complex (SOC). In the E1.6 group, significant decrements of BDNF immunoreactivity (IR) were noted in the lateral superior olive, medial superior olive, superior paraolivary nucleus and medial nucleus of the trapezoid body. GDNF IR was also significantly decreased ($p < 0.001$) in all SOC nuclei of the E1.6 group. The decrease in the IR of these neurotrophic factors in the SOC of the E1.6 group suggests a detrimental effect of RF exposure in the auditory nuclei.

(E) Mathur R. Effect of chronic intermittent exposure to AM radiofrequency field on responses to various types of noxious stimuli in growing rats. Electromagn Biol Med. 27(3):266-276, 2008. (AS, CE, BE)

There are several reports of altered pain sensation after exposure (from a few minutes to hours in single or repeated doses for 2-3 weeks) to electromagnetic fields (EMF) in adults. The commonly utilized noxious stimulus is radiant heat. The nociceptive responses are known to be influenced by characteristics of stimulus, organism, and environment. We studied the pattern of nociceptive responses to various noxious stimuli in growing rats exposed to radiofrequency field (73.5 MHz amplitude modulated, 16 Hz power density 1.33 mw/cm(2), SAR = 0.4 w/kg) for 45 d (2 h/d). Threshold current for stimulation of nociceptive afferents to mediate motor response of tail (TF), vocalization during stimulus (VD), and vocalization after discharge (VA); the withdrawal latency of tail (TFL) and hind paw (HPL) to thermal noxious stimulus and tonic pain responses were recorded in every rat. The TFL was not affected, HPL was decreased ($p < 0.01$), and the thresholds of TF and VD were not affected, while, that of VA was significantly decreased. The tonic pain rating was decreased ($p < 0.01$). A decrease in the threshold of VA ($p < 0.01$) is indicative of an increase in the emotional component of the response to the phasic pain, whereas a decrease in the pain rating indicates analgesia in response to the tonic pain. The results of our study suggest that chronic (45 d), intermittent (2 h/d) amplitude modulated RF field exposure to the peripubertal rat increases the emotional component of phasic pain over a basal eaualgesic state, while late response to tonic pain is decreased. The data suggest that amplitude modulated RF field differentially affects the mechanisms involved in the processing of various noxious stimuli.

(E) Megha K, Deshmukh PS, Banerjee BD, Tripathi AK, Abegaonkar MP. Microwave radiation induced oxidative stress, cognitive impairment and inflammation in brain of Fischer rats. Indian J Exp Biol. 50(12):889-896, 2012. (AS, LI, CE, BE, OX, CH)

Public concerns over possible adverse effects of microwave radiation emitted by mobile phones on health are increasing. To evaluate the intensity of oxidative stress, cognitive impairment and inflammation in brain of Fischer rats exposed to microwave radiation, male Fischer-344 rats were exposed to 900 MHz microwave radiation (SAR = 5.953 x 10(-4) W/kg) and 1800 MHz microwave radiation (SAR = 5.835 x 10(-4) W/kg) for 30 days (2 h/day). Significant impairment in cognitive function and induction of oxidative stress in brain tissues of microwave exposed rats were observed in comparison with sham exposed groups. Further, significant increase in level of cytokines (IL-6 and TNF-alpha) was also observed following microwave exposure. Results of the present study indicated that increased oxidative stress due to microwave exposure may contribute to cognitive impairment and inflammation in brain.

(E) Meral I, Mert H, Mert N, Deger Y, Yoruk I, Yetkin A, Keskin S. Effects of 900-MHz electromagnetic field emitted from cellular phone on brain oxidative stress and some vitamin levels of guinea pigs. Brain Res. 1169:120-124, 2007. (AS, CE, OX)

This study was designed to demonstrate the effects of 900-MHz electromagnetic field (EMF) emitted from cellular phone on brain tissue and also blood malondialdehyde (MDA), glutathione (GSH), retinol (vitamin A), vitamin D(3) and tocopherol (vitamin E) levels, and catalase (CAT) enzyme activity of guinea pigs. Fourteen male guinea pigs, weighing 500-800 g were randomly divided into one of two experimental groups: control and treatment (EMF-exposed), each containing seven animals. Animals in treatment group were exposed to 890- to 915-MHz EMF (217-Hz pulse rate, 2-W maximum peak power, SAR 0.95 w/kg) of a cellular phone for 12 h/day (11-h 45-min stand-by and 15-min spiking mode) for 30 days. Control guinea pigs were housed in a separate room without exposing EMF of a cellular phone. Blood samples were collected through a cardiac puncture and brains were removed after decapitation for the biochemical analysis at the end of the 30 days of experimental period. It was found that the MDA level increased (P<0.05), GSH level and CAT enzyme activity decreased (P<0.05), and vitamins A, E and D(3) levels did not change (P>0.05) in the brain tissues of EMF-exposed guinea pigs. In addition, MDA, vitamins A, D(3) and E levels, and CAT enzyme activity increased (P<0.05), and GSH level decreased (P<0.05) in the blood of EMF-exposed guinea pigs. It was concluded that electromagnetic field emitted from cellular phone might produce oxidative stress in brain tissue of guinea pigs. However, more studies are needed to demonstrate whether these effects are harmful or/and affect the neural functions.

(NE) Mohler E, Frei P, Braun-Fahrländer C, Fröhlich J, Neubauer G, Röösli M; Qualifex Team. Effects of everyday radiofrequency electromagnetic-field exposure on sleep quality: a cross-sectional study. Radiat Res. 174(3):347-356, 2010. (HU, SL)

The aim of this cross-sectional study was to investigate the association between exposure to various sources of radiofrequency electromagnetic fields (RF EMFs) in the everyday environment and sleep quality, which is a common public health concern. We assessed self-reported sleep disturbances and daytime sleepiness in a random population sample of 1,375 inhabitants from the area of Basel, Switzerland. Exposure to environmental far-field RF EMFs was predicted for each individual using a prediction model that had been developed and validated previously. Self-reported cordless and mobile phone use as well as objective mobile phone operator data for the previous 6 months were also considered in the analyses. In multivariable regression models, adjusted for relevant confounders, no associations between environmental far-field RF EMF exposure and sleep disturbances or excessive daytime sleepiness were observed. The 10% most exposed participants had an estimated risk for sleep disturbances of 1.11 (95% CI: 0.50 to 2.44) and for excessive daytime sleepiness of 0.58 (95% CI: 0.31 to 1.05). Neither mobile phone use nor cordless phone use was associated with decreased sleep quality. The results of this large cross-sectional study did not indicate an impairment of subjective sleep quality due to exposure from various sources of RF EMFs in everyday life.

(NE) Mohler E, Frei P, Fröhlich J, Braun-Fahrländer C, Röösli M; QUALIFEX-team. Exposure to radiofrequency electromagnetic fields and sleep quality: a prospective cohort study. PLoS One. 7(5):e37455, 2012. (HU, SL)

BACKGROUND: There is persistent public concern about sleep disturbances due to radiofrequency electromagnetic field (RF-EMF) exposure. The aim of this prospective cohort study was to investigate whether sleep quality is affected by mobile phone use or by other RF-EMF sources in the everyday environment. METHODS: We conducted a prospective cohort study with 955 study participants aged between 30 and 60 years. Sleep quality and daytime sleepiness was assessed by means of standardized questionnaires in May 2008 (baseline) and May 2009 (follow-up). We also asked about mobile and cordless phone use and asked study participants for consent to obtain their mobile phone connection data from the mobile phone operators. Exposure to environmental RF-EMF was computed for each study participant using a previously developed and validated prediction model. In a nested sample of 119 study participants, RF-EMF exposure was measured in the bedroom and data on sleep behavior was collected by means of actigraphy during two weeks. Data were analyzed using multivariable regression models adjusted for relevant confounders. RESULTS: In the longitudinal analyses neither operator-recorded nor self-reported mobile phone use was associated with sleep disturbances or daytime sleepiness. Also, exposure to environmental RF-EMF did not affect self-reported sleep quality. The results from the longitudinal analyses were confirmed in the nested sleep study with objectively recorded exposure and measured sleep behavior data. CONCLUSIONS: We did not find evidence for adverse effects on sleep quality from RF-EMF exposure in our everyday environment.

(E) Mohammed HS, Fahmy HM, Radwah NM, Elsayed AA. Non-thermal continuous and modulated electromagnetic radiation fields effects on sleep EEG of rats. J Adv Res 4(2) 181-187, 2013. (AS, EE, SL, WS)

In the present study, the alteration in the sleep EEG in rats due to chronic exposure to low-level non-thermal electromagnetic radiation was investigated. Two types of radiation fields were used; 900 MHz *unmodulated* wave and 900 MHz *modulated* at 8 and 16 Hz waves. Animals has exposed to radiation fields for 1 month (1 h/day). EEG power spectral analyses of exposed and control animals during slow wave sleep (SWS) and rapid eye movement sleep (REM sleep) revealed that the REM sleep is more susceptible to modulated radiofrequency radiation fields (RFR) than the SWS. The latency of REM sleep increased due to radiation exposure indicating a change in the ultradian rhythm of normal sleep cycles. The cumulative and irreversible effect of radiation exposure was proposed and the interaction of the extremely low frequency radiation with the similar EEG frequencies was suggested.

(E) Moretti D, Garenne A, Haro E, Poulletier de Gannes F, Lagroye I, Lévêque P, Veyret B, Lewis N. In-vitro exposure of neuronal networks to the GSM-1800 signal. Bioelectromagnetics. 2013 Aug 1. doi: 10.1002/bem.21805. [Epub ahead of print] (CS, EE)

The central nervous system is the most likely target of mobile telephony radiofrequency (RF) field exposure in terms of biological effects. Several electroencephalography (EEG) studies have reported variations in the alpha-band power spectrum during and/or after RF exposure, in resting EEG and during sleep. In this context, the observation of the spontaneous electrical activity of neuronal networks under RF exposure can be an efficient tool to detect the occurrence of low-level RF effects on the nervous system. Our research group has developed a dedicated experimental setup in the GHz range for the simultaneous exposure of neuronal networks and monitoring of electrical activity. A transverse electromagnetic (TEM) cell was used to expose the neuronal networks to GSM-1800 signals at a SAR level of 3.2 W/kg. Recording of the neuronal electrical activity and detection of the extracellular spikes and bursts under exposure were performed using microelectrode arrays (MEAs). This work provides the proof of feasibility and preliminary results of the integrated investigation regarding exposure setup, culture of the neuronal network, recording of the electrical activity, and analysis of the signals obtained under RF exposure. In this pilot study on 16 cultures, there was a 30% reversible decrease in firing rate (FR) and bursting rate (BR) during a 3 min exposure to RF. Additional experiments are needed to further characterize this effect.

(NE) Nakatani-Enomoto S, Furubayashi T, Ushiyama A, Groiss SJ, Ueshima K, Sokejima S, Simba AY, Wake K, Watanabe SI, Nishikawa M, Miyawaki K, Taki M, Ugawa Y. Effects of electromagnetic fields emitted from W-CDMA-like mobile phones on sleep in humans. Bioelectromagnetics. 2013 Aug 22. doi: 10.1002/bem.21809. [Epub ahead of print] (HU, EE, SL)

In this study, we investigated subjective and objective effects of mobile phones using a Wideband Code Division Multiple Access (W-CDMA)-like system on human sleep. Subjects were 19 volunteers. Real or sham electromagnetic field (EMF) exposures for 3 h were performed before their usual sleep time on 3 consecutive days. They were exposed to real EMF on the second or third experimental day in a double-blind design. Sleepiness and sleep insufficiency were evaluated the next morning. Polysomnograms were recorded for analyses of the sleep variables and power spectra of electroencephalograms (EEG). No significant differences were observed between the two conditions in subjective feelings. Sleep parameters including sleep stage percentages and EEG power spectra did not differ significantly between real and sham exposures. We conclude that continuous wave EMF exposure for 3 h from a W-CDMA-like system has no detectable effects on human sleep.

(E) Narayanan SN, Kumar RS, Potu BK, Nayak S, Mailankot M. Spatial memory performance of Wistar rats exposed to mobile phone. Clinics (Sao Paulo). 64(3):231-234, 2009. (AS, CE, BE)

INTRODUCTION: With the tremendous increase in number of mobile phone users world wide, the possible risks of this technology have become a serious concern. OBJECTIVE: We tested the effects of mobile phone exposure on spatial memory performance. MATERIALS AND METHODS: Male Wistar rats (10-12 weeks old) were exposed to 50 missed calls/day for 4 weeks from a GSM (900/1800 MHz) mobile phone in vibratory mode (no ring tone). After the experimental period, the animals were tested for spatial memory performance using the Morris water maze test. RESULTS: Both phone exposed and control animals showed a significant decrease in escape time with training. Phone exposed animals had significantly (approximately 3 times) higher mean latency to reach the target quadrant and spent significantly (approximately 2 times) less time in the target quadrant than age- and sex-matched controls. CONCLUSION: Mobile phone exposure affected the acquisition of learned responses in Wistar rats. This in turn points to the poor spatial navigation and the object place configurations of the phone-exposed animals.

(E) Narayanan SN, Kumar RS, Potu BK, Nayak S, Bhat PG, Mailankot M. Effect of radio-frequency electromagnetic radiations (RF-EMR) on passive avoidance behaviour and hippocampal morphology in Wistar rats. Ups J Med Sci. 115(2):91-96, 2010. (AS, CE, ME, BE)

INTRODUCTION: The interaction of mobile phone radio-frequency electromagnetic radiation (RF-EMR) with the brain is a serious concern of our society. **OBJECTIVE:** We evaluated the effect of RF-EMR from mobile phones on passive avoidance behaviour and hippocampal morphology in rats. **MATERIALS AND METHODS:** Healthy male albino Wistar rats were exposed to RF-EMR by giving 50 missed calls (within 1 hour) per day for 4 weeks, keeping a GSM (0.9 GHz/1.8 GHz) mobile phone in vibratory mode (no ring tone) in the cage. After the experimental period, passive avoidance behaviour and hippocampal morphology were studied. **RESULTS:** Passive avoidance behaviour was significantly affected in mobile phone RF-EMR-exposed rats demonstrated as shorter entrance latency to the dark compartment when compared to the control rats. Marked morphological changes were also observed in the CA(3) region of the hippocampus of the mobile phone-exposed rats in comparison to the control rats. **CONCLUSION:** Mobile phone RF-EMR exposure significantly altered the passive avoidance behaviour and hippocampal morphology in rats.

(E) Narayanan SN, Kumar RS, Paval J, Kedage V, Bhat MS, Nayak S, Bhat PG. Analysis of emotionality and locomotion in radio-frequency electromagnetic radiation exposed rats. Neurol Sci. 34(7):1117-1124, 2013. (AS, CE, BE)

In the current study the modulatory role of mobile phone radio-frequency electromagnetic radiation (RF-EMR) on emotionality and locomotion was evaluated in adolescent rats. Male albino Wistar rats (6-8 weeks old) were randomly assigned into the following groups having 12 animals in each group. Group I (Control): they remained in the home cage throughout the experimental period. Group II (Sham exposed): they were exposed to mobile phone in switch-off mode for 28 days, and Group III (RF-EMR exposed): they were exposed to RF-EMR (900 MHz) from an active GSM (Global system for mobile communications) mobile phone with a peak power density of 146.60 μW/cm(2) for 28 days. On 29th day, the animals were tested for emotionality and locomotion. Elevated plus maze (EPM) test revealed that, percentage of entries into the open arm, percentage of time spent on the open arm and distance travelled on the open arm were significantly reduced in the RF-EMR exposed rats. Rearing frequency and grooming frequency were also decreased in the RF-EMR exposed rats. Defecation boli count during the EPM test was more with the RF-EMR group. No statistically significant difference was found in total distance travelled, total arm entries, percentage of closed arm entries and parallelism index in the RF-EMR exposed rats compared to controls. Results indicate that mobile phone radiation could affect the emotionality of rats without affecting the general locomotion.

(E) Nazıroğlu M, Çelik Ö, Özgül C, Çiğ B, Doğan S, Bal R, Gümral N, Rodríguez AB, Pariente JA. Melatonin modulates wireless (2.45 GHz)-induced oxidative injury through TRPM2 and voltage gated Ca(2+) channels in brain and dorsal root ganglion in rat. Physiol Behav. 105(3):683-692, 2012. (AS, CE, CH, EE, OX)

We aimed to investigate the protective effects of melatonin and 2.45 GHz electromagnetic radiation (EMR) on brain and dorsal root ganglion (DRG) neuron antioxidant redox system, Ca(2+) influx, cell viability and electroencephalography (EEG) records in the rat. Thirty two rats were equally divided into four different groups namely group A1: Cage control, group A2: Sham control, group B: 2.45 GHz EMR, group C: 2.45 GHz EMR+melatonin. Groups B and C were exposed to 2.45 GHz EMR during 60 min/day for 30 days. End of the experiments, EEG records and the brain cortex and DRG samples were taken. Lipid peroxidation (LP), cell viability and cytosolic Ca(2+) values in DRG neurons were higher in group B than in groups A1 and A2 although their concentrations were increased by melatonin, 2-aminoethyldiphenyl borinate (2-APB), diltiazem and verapamil supplementation. Spike numbers of EEG records in group C were lower than in group B. Brain cortex vitamin E concentration was higher in group C than in group B. In conclusion, Melatonin supplementation in DRG neurons and brain seems to have protective effects on the 2.45 GHz-induced increase Ca(2+) influx, EEG records and cell viability of the hormone through TRPM2 and voltage gated Ca(2+) channels.

(E) Ning W, Xu SJ, Chiang H, Xu ZP, Zhou SY, Yang W, Luo JH. Effects of GSM 1800 MHz on dendritic development of cultured hippocampal neurons. Acta Pharmacol Sin. 28(12):1873-1880, 2007. (CS, CE, DE, ME)

AIM: To evaluate the effects of global system for mobile communications (GSM) 1800 MHz microwaves on dendritic filopodia, dendritic arborization, and spine maturation during development in cultured hippocampal neurons in rats. **METHODS:** The cultured hippocampal neurons were exposed to GSM 1800 MHz microwaves with 2.4 and 0.8 W/kg, respectively, for 15 min each day from 6 days in vitro (DIV6) to DIV14. The subtle structures of dendrites were displayed by transfection with farnesylated enhanced green fluorescent protein (F-GFP) and GFP-actin on DIV5 into the hippocampal neurons. **RESULTS:** There was a significant decrease in the density and mobility of dendritic filopodia at DIV8 and in the density of mature spines at DIV14 in the neurons exposed to GSM 1800 MHz microwaves with 2.4 W/kg. In addition, the average length of dendrites per neuron at DIV10 and DIV14 was decreased, while the dendritic arborization was unaltered in these neurons. However, there were no significant changes found in the neurons exposed to the GSM 1800 MHz microwaves with 0.8 W/kg. **CONCLUSION:** These data indicate that the chronic exposure to 2.4 W/kg GSM 1800 MHz microwaves during the early developmental stage may affect dendritic development and the formation of excitatory synapses of hippocampal neurons in culture.

(E) Nittby H, Widegren B, Krogh M, Grafström G, Berlin H, Rehn G, Eberhardt JL, Malmgren L, Persson BRR, Salford L. Exposure to radiation from global system for mobile communications at 1,800 MHz significantly changes gene expression in rat hippocampus and cortex. Environmentalist 28(4), 458-465, 2008. (AS, CH, LI)

We have earlier shown that radio frequency electromagnetic fields can cause significant leakage of albumin through the blood–brain barrier of exposed rats as compared to non-exposed rats, and also significant neuronal damage in rat brains several weeks after a 2 h exposure to a mobile phone, at 915 MHz with a global system for mobile communications (GSM) frequency modulation, at whole-body specific absorption rate values (SAR) of 200, 20, 2, and 0.2 mW/kg. We have now studied whether 6 h of exposure to the radiation from a GSM mobile test phone at 1,800 MHz (at a whole-body SAR-value of 13 mW/kg, corresponding to a brain SAR-value of 30 mW/kg) has an effect upon the gene expression pattern in rat brain cortex and hippocampus—areas where we have observed albumin leakage from capillaries into neurons and neuronal damage. Microarray analysis of 31,099 rat genes, including splicing variants, was performed in cortex and hippocampus of 8 Fischer 344 rats, 4 animals exposed to global system for mobile communications electromagnetic fields for 6 h in an anechoic chamber, one rat at a time, and 4 controls kept as long in the same anechoic chamber without exposure, also in this case one rat at a time. Gene ontology analysis (using the gene ontology categories biological processes, molecular functions, and cell components) of the differentially expressed genes of the exposed animals versus the control group revealed the following highly significant altered gene categories in both cortex and hippocampus: extracellular region, signal transducer activity, intrinsic to membrane, and integral to membrane. The fact that most of these categories are connected with membrane functions may have a relation to our earlier observation of albumin transport through brain capillaries.

(E) Nittby H, Grafström G, Tian DP, Malmgren L, Brun A, Persson BR, Salford LG, Eberhardt J. Cognitive impairment in rats after long-term exposure to GSM-900 mobile phone radiation. Bioelectromagnetics. 29(3):219-232, 2008. (AS, CE, BE, LI)

Considering the frequent use of mobile phones, we have directed attention to possible implications on cognitive functions. In this study we investigated in a rat model the long-term effects of protracted exposure to Global System for Mobile Communication-900 MHz (GSM-900) radiation. Out of a total of 56 rats, 32 were exposed for 2 h each week for 55 weeks to radio-frequency electromagnetic radiation at different SAR levels (0.6 and 60 mW/kg at the initiation of the experimental period) emitted by a (GSM-900) test phone. Sixteen animals were sham exposed and eight animals were cage controls, which never left the animal house. After this protracted exposure, GSM-900 exposed rats were compared to sham exposed controls. Effects on exploratory behaviour were evaluated in the open-field test, in which no difference was seen. Effects on cognitive functions were evaluated in the episodic-like memory test. In our study, GSM exposed rats had impaired memory for objects and their temporal order of presentation, compared to sham exposed controls (P = 0.02). Detecting the place in which an object was presented was not affected by GSM exposure. Our results suggest significantly reduced memory functions in rats after GSM microwave exposure (P = 0.02).

(E) Nittby H, Brun A, Eberhardt J, Malmgren L, Persson BR, Salford LG. Increased blood-brain barrier permeability in mammalian brain 7 days after exposure to the

radiation from a GSM-900 mobile phone. Pathophysiology. 16(2-3):103-112, 2009. (AS, ME, LI)

Microwaves were for the first time produced by humans in 1886 when radio waves were broadcasted and received. Until then microwaves had only existed as a part of the cosmic background radiation since the birth of universe. By the following utilization of microwaves in telegraph communication, radars, television and above all, in the modern mobile phone technology, mankind is today exposed to microwaves at a level up to 10(20) times the original background radiation since the birth of universe. Our group has earlier shown that the electromagnetic radiation emitted by mobile phones alters the permeability of the blood-brain barrier (BBB), resulting in albumin extravasation immediately and 14 days after 2h of exposure. In the background section of this report, we present a thorough review of the literature on the demonstrated effects (or lack of effects) of microwave exposure upon the BBB. Furthermore, we have continued our own studies by investigating the effects of GSM mobile phone radiation upon the blood-brain barrier permeability of rats 7 days after one occasion of 2h of exposure. Forty-eight rats were exposed in TEM-cells for 2h at non-thermal specific absorption rates (SARs) of 0mW/kg, 0.12mW/kg, 1.2mW/kg, 12mW/kg and 120mW/kg. Albumin extravasation over the BBB, neuronal albumin uptake and neuronal damage were assessed. Albumin extravasation was enhanced in the mobile phone exposed rats as compared to sham controls after this 7-day recovery period (Fisher's exact probability test, p=0.04 and Kruskal-Wallis, p=0.012), at the SAR-value of 12mW/kg (Mann-Whitney, p=0.007) and with a trend of increased albumin extravasation also at the SAR-values of 0.12mW/kg and 120mW/kg. There was a low, but significant correlation between the exposure level (SAR-value) and occurrence of focal albumin extravasation (r(s)=0.33; p=0.04). The present findings are in agreement with our earlier studies where we have seen increased BBB permeability immediately and 14 days after exposure. We here discuss the present findings as well as the previous results of altered BBB permeability from our and other laboratories.

(E) Nittby H, Moghadam MK, Sun W, Malmgren L, Eberhardt J, Persson BR, Salford LG. Analgetic effects of non-thermal GSM-1900 radiofrequency electromagnetic fields in the land snail Helix pomatia. Int J Radiat Biol. 88(3):245-252, 2012. (AS, BE, MA, LI)

PURPOSE: To investigate whether mobile phone radiation might affect snail nociception, employing radiofrequency (RF) electromagnetic fields (EMF) which, to our knowledge, have hitherto not been studied in a snail model. Exposure to extremely low frequency (ELF) magnetic fields has however been shown to significantly affect nociceptive responses. MATERIALS AND METHODS: In the present study, we exposed 29 land snails of the strain Helix pomatia to global system for mobile communications (GSM) EMF at 1900 MHz at the non-thermal level 48 mW/kg for 1 hour each and 29 snails were sham controls. The experiments took place during the onset of summer, with all snails being well out of hibernation. Before and after GSM or sham exposure, the snails were subjected to thermal pain by being placed on a hot plate. The reaction time for retraction from the hot plate was measured by two blinded observers. RESULTS: Comparing the reaction pattern of each snail before and after exposure, the GSM-exposed snails were less sensitive to thermal pain as compared to the sham controls, indicating that RF exposure induces a significant analgesia (Mann-Whitney p < 0.001). CONCLUSION: This study might support earlier findings, describing beneficial effects of EMF exposure upon nociception.

(E) Noor NA, Mohammed HS, Ahmed NA, Radwan NM. Variations in amino acid neurotransmitters in some brain areas of adult and young male albino rats due to exposure to mobile phone radiation. Eur Rev Med Pharmacol Sci. 15(7):729-742, 2011. (AS, CE, CH, AD)

BACKGROUND AND OBJECTIVES: Mobile phone radiation and health concerns have been raised, especially following the enormous increase in the use of wireless mobile telephony throughout the world. The present study aims to investigate the effect of one hour daily exposure to electromagnetic radiation (EMR) with frequency of 900 Mz (SAR 1.165 w/kg, power density 0.02 mW/cm2) on the levels of amino acid neurotransmitters in the midbrain, cerebellum and medulla of adult and young male albino rats. **MATERIALS AND METHODS:** Adult and young rats were divided into two main groups (treated and control). The treated group of both adult and young rats was exposed to EMR for 1 hour daily. The other group of both adult and young animals was served as control. The determination of amino acid levels was carried out after 1 hour, 1 month, 2 months and 4 months of EMR exposure as well as after stopping radiation. **RESULTS:** Data of the present study showed a significant increase in both excitatory and inhibitory amino acids in the cerebellum of adult and young rats and midbrain of adult animals after 1 hour of EMR exposure. In the midbrain of adult animals, there was a significant increase in glycine level after 1 month followed by significant increase in GABA after 4 months. Young rats showed significant decreases in the midbrain excitatory amino acids. In the medulla, the equilibrium ratio percent (ER%) calculations showed a state of neurochemical inhibition after 4 months in case of adult animals, whereas in young animals, the neurochemical inhibitory state was observed after 1 month of exposure due to significant decrease in glutamate and aspartate levels. This state was converted to excitation after 4 months due to the increase in glutamate level. **CONCLUSION:** The present changes in amino acid concentrations may underlie the reported adverse effects of using mobile phones.

(E) Ntzouni MP, Stamatakis A, Stylianopoulou F, Margaritis LH. Short-term memory in mice is affected by mobile phone radiation. Pathophysiology. 18(3):193-199, 2011. (AS, CE, BE)

The effects of mobile phone electromagnetic fields (EMFs) were studied on a non-spatial memory task (Object Recognition Task - ORT) that requires entorhinal cortex function. The task was applied to three groups of mice Mus musculus C57BL/6 (exposed, sham-exposed and control) combined with 3 different radiation exposure protocols. In the first protocol designated "acute exposure", mice 45 days old (PND45 - postnatal day 45) were exposed to mobile phone (MP) radiation (SAR value 0.22W/kg) during the habituation, the training and the test sessions of the ORT, but not during the 10min inter-trial interval (ITI) where consolidation of stored object information takes place. On the second protocol designated "chronic exposure-I", the same mice were exposed for 17 days for 90min/per day starting at PND55 to the same MP radiation. ORT recognition memory was performed at PND72 with radiation present only during the ITI phase. In the third protocol designated "chronic exposure-II", mice continued to be exposed daily under the same conditions up to PND86 having received radiation for 31 days. One day later the ORT test was performed without irradiation present in any of the sessions. The ORT-derived discrimination indices in all three exposure protocols revealed a major effect on the "chronic exposure-I" suggesting a possible severe interaction of EMF with the consolidation phase of recognition memory processes. This may imply that the primary EMF target may be the information transfer pathway connecting the entorhinal-parahippocampal regions which participate in the ORT memory task.

(E) Ntzouni MP, Skouroliakou A, Kostomitsopoulos N, Margaritis LH. Transient and cumulative memory impairments induced by GSM 1.8 GHz cell phone signal in a mouse model. Electromagn Biol Med. 2013 Jan 15. [Epub ahead of print] (AS, CE, BE)

This study was designed to investigate the transient and cumulative impairments in spatial and non-spatial memory of C57Bl/6J mice exposed to GSM 1.8 GHz signal for 90 min daily by a typical cellular (mobile) phone at a specific absorption rate value of 0.11 W/kg. Free-moving male mice 2 months old were irradiated in two experimental protocols, lasting for 66 and for 148 days respectively. Each protocol used three groups of animals (n = 8 each for exposed, sham exposed and controls) in combination with two behavioural paradigms, the object recognition task and the object location task sequentially applied at different time points. One-way analysis of variance revealed statistically significant impairments of both types of memory gradually accumulating, with more pronounced effects on the spatial memory. The impairments persisted even 2 weeks after interruption of the 8 weeks daily exposure, whereas the memory of mice as detected by both tasks showed a full recovery approximately 1 month later. Intermittent every other day exposure for 1 month had no effect on both types of memory. The data suggest that visual information processing mechanisms in hippocampus, perirhinal and entorhinal cortex are gradually malfunctioning upon long-term daily exposure, a phenotype that persists for at least 2 weeks after interruption of radiation, returning to normal memory performance levels 4 weeks later. It is postulated that cellular repair mechanisms are operating to eliminate the memory affecting molecules. The overall contribution of several possible mechanisms to the observed cumulative and transient impairments in spatial and non-spatial memory is discussed.

(NE) Nylund R, Kuster N, Leszczynski D. Analysis of proteome response to the mobile phone radiation in two types of human primary endothelial cells. Proteome Sci. 8:52, 2010. (CS, CH, WS)

BACKGROUND: Use of mobile phones has widely increased over the past decade. However, in spite of the extensive research, the question of potential health effects of the mobile phone radiation remains unanswered. We have earlier proposed, and applied, proteomics as a tool to study biological effects of the mobile phone radiation, using as a model human endothelial cell line EA.hy926. Exposure of EA.hy926 cells to 900 MHz GSM radiation has caused statistically significant changes in expression of numerous proteins. However, exposure of EA.hy926 cells to 1800 MHz GSM signal had only very small effect on cell proteome, as compared with 900 MHz GSM exposure. In the present study, using as model human primary endothelial cells, we have examined whether exposure to 1800 MHz GSM mobile phone radiation can affect cell proteome.
RESULTS: Primary human umbilical vein endothelial cells and primary human brain microvascular endothelial cells were exposed for 1 hour to 1800 MHz GSM mobile phone radiation at an average specific absorption rate of 2.0 W/kg. The cells were harvested immediately after the exposure and the protein expression patterns of the sham-exposed and radiation-exposed cells were examined using two dimensional difference gel electrophoresis-based proteomics (2DE-DIGE). There were observed numerous differences between the proteomes of human umbilical vein endothelial cells and human brain microvascular endothelial cells (both sham-exposed). These differences are most likely representing

physiological differences between endothelia in different vascular beds. However, the exposure of both types of primary endothelial cells to mobile phone radiation did not cause any statistically significant changes in protein expression. **CONCLUSIONS:** Exposure of primary human endothelial cells to the mobile phone radiation, 1800 MHz GSM signal for 1 hour at an average specific absorption rate of 2.0 W/kg, does not affect protein expression, when the proteomes were examined immediately after the end of the exposure and when the false discovery rate correction was applied to analysis. This observation agrees with our earlier study showing that the 1800 MHz GSM radiation exposure had only very limited effect on the proteome of human endothelial cell line EA.hy926, as compared with the effect of 900 MHz GSM radiation.

(NE) O'Connor RP, Madison SD, Leveque P, Roderick HL, Bootman MD. Exposure to GSM RF fields does not affect calcium homeostasis in human endothelial cells, rat pheocromocytoma cells or rat hippocampal neurons. PLoS One. 5(7):e11828, 2010. (CS, CC, CH)

In the course of modern daily life, individuals are exposed to numerous sources of electromagnetic radiation that are not present in the natural environment. The strength of the electromagnetic fields from sources such as hairdryers, computer display units and other electrical devices is modest. However, in many home and office environments, individuals can experience perpetual exposure to an "electromagnetic smog", with occasional peaks of relatively high electromagnetic field intensity. This has led to concerns that such radiation can affect health. In particular, emissions from mobile phones or mobile phone masts have been invoked as a potential source of pathological electromagnetic radiation. Previous reports have suggested that cellular calcium (Ca2+) homeostasis is affected by the types of radiofrequency fields emitted by mobile phones. In the present study, we used a high-throughput imaging platform to monitor putative changes in cellular Ca2+ during exposure of cells to 900 MHz GSM fields of differing power (specific absorption rate 0.012-2 W/Kg), thus mimicking the type of radiation emitted by current mobile phone handsets. Data from cells experiencing the 900 Mhz GSM fields were compared with data obtained from paired experiments using continuous wave fields or no field. We employed three cell types (human endothelial cells, PC-12 neuroblastoma and primary hippocampal neurons) that have previously been suggested to be sensitive to radiofrequency fields. Experiments were designed to examine putative effects of radiofrequency fields on resting Ca2+, in addition to Ca2+ signals evoked by an InsP(3)-generating agonist. Furthermore, we examined putative effects of radiofrequency field exposure on Ca2+ store emptying and store-operated Ca2+ entry following application of the Ca2+ATPase inhibitor thapsigargin. Multiple parameters (e.g., peak amplitude, integrated Ca2+ signal, recovery rates) were analysed to explore potential impact of radiofrequency field exposure on Ca2+ signals. Our data indicate that 900 MHz GSM fields do not affect either basal Ca2+ homeostasis or provoked Ca2+ signals. Even at the highest field strengths applied, which exceed typical phone exposure levels, we did not observe any changes in cellular Ca2+ signals. We conclude that under the conditions employed in our experiments, and using a highly-sensitive assay, we could not detect any consequence of RF exposure.

(E) Odaci E, Bas O, Kaplan S. Effects of prenatal exposure to a 900 MHz electromagnetic field on the dentate gyrus of rats: a stereological and histopathological study. Brain Res. 1238:224-229, 2008. (AS, CE, DE, ME)

Electromagnetic fields (EMFs) inhibit the formation and differentiation of neural stem cells during embryonic development. In this study, the effects of prenatal exposure to EMF on the number of granule cells in the dentate gyrus of 4-week-old rats were investigated. This experiment used a control (Cont) group and an EMF exposed (EMF) group (three pregnant rats each group). The EMF group consisted of six offspring (n=6) of pregnant rats that were exposed to an EMF of up to 900 megahertz (MHz) for 60 min/day between the first and last days of gestation. The control group consisted of five offspring (n=5) of pregnant rats that were not treated at all. The offspring were sacrificed when they were 4 weeks old. The numbers of granule cells in the dentate gyrus were analyzed using the optical fractionator technique. The results showed that prenatal EMF exposure caused a decrease in the number of granule cells in the dentate gyrus of the rats (P<0.01). This suggests that prenatal exposure to a 900 MHz EMF affects the development of the dentate gyrus granule cells in the rat hippocampus. Cell loss might be caused by an inhibition of granule cell neurogenesis in the dentate gyrus.

(E) Odacı E, İkinci A, Yıldırım M, Kaya H, Akça M, Hancı H, Sönmez OF, Aslan A, Okuyan M, Baş O. The Effects of 900 Megahertz Electromagnetic Field Applied in the Prenatal Period on Spinal Cord Morphology and Motor Behavior in Female Rat Pups. NeuroQuantology 11:573-581, 2013. (AS, CE, DE, BE, ME)

This study investigated the effect of a 900 megahertz (MHz) electromagnetic field (EMF) applied in the prenatal period on the spinal cord and motor behavior of female rat pups. Beginning of the study, female Sprague Dawley rats (180–250 g) were left to mate with male rats. Rats identified as pregnant were then divided into control (n=3) and EMF groups (n=3). The EMF group was exposed to 1-h 900 MHz EMF daily between days 13 and 21 of pregnancy. At 21 days old, rat pups were removed from their mothers and divided into two newborn rat groups, control (n=13) and EMF (n=10). The rotarod test was applied to the rat pups to assess motor functions and the open field test to evaluate locomotor activity. On day 32 of the study, the rat pups were decapitated, and the spinal cord in the upper thoracic region was removed. Following routine histological tests, they were stained with Cresyl fast violet. Rotarod test results revealed a significant increase in EMF group rat pups' motor functions (p=0.037). However, no difference was observed in the open field test results (p>0.05). In the EMF group' rat pups, we observed pathological changes in the spinal cord. On the basis of our results, 900 MHz EMF applied in the prenatal period affected spinal cord development. This effect was observed in the form of pathological changes in the spinal cord of rat pups, and it may be that these pathological changes led to an increase in rat pups' motor activities.

(NE) Ogawa K, Nabae K, Wang J, Wake K, Watanabe S, Kawabe M, Fujiwara O, Takahashi S, Ichihara T, Tamano S, Shirai T. Effects of gestational exposure to 1.95-GHz W-CDMA signals for IMT-2000 cellular phones: Lack of embryotoxicity and teratogenicity in rats. Bioelectromagnetics. 30(3):205-212, 2009. (AS, CE, DE)

The present study was designed to evaluate whether gestational exposure to an EMF targeting the head region, similar to that from cellular phones, might affect embryogenesis in rats. A 1.95-GHz wide-band code division multiple access (W-CDMA) signal, which is one applied for the International Mobile Telecommunication 2000 (IMT-2000) system and used for the freedom of mobile multimedia access (FOMA), was employed for exposure to the heads of four groups of pregnant CD(SD) IGS rats (20 per group) for gestational days 7-17. The exposure was performed for 90 min/day in the morning. The spatial average specific absorption rate (SAR) for individual brains was designed to be 0.67 and 2.0 W/kg with peak brain SARs of 3.1 and 7.0 W/kg for low (group 3) and high (group 4) exposures, respectively, and a whole-body average SAR less than 0.4 W/kg so as not to cause thermal effects due to temperature elevation. Control and sham exposure groups were also included. At gestational day 20, all dams were killed and fetuses were taken out by cesarean section. There were no differences in maternal body weight gain. No adverse effects of EMF exposure were observed on any reproductive and embryotoxic parameters such as number of live (243-271 fetuses), dead or resorbed embryos, placental weights, sex ratios, weights or external, visceral or skeletal abnormalities of live fetuses.

(NE) Okano T, Terao Y, Furubayashi T, Yugeta A, Hanajima R, Ugawa Y. The effect of electromagnetic field emitted by a mobile phone on the inhibitory control of saccades. Clin Neurophysiol. 121(4):603-611, 2010. (HU, PE)

OBJECTIVE: To investigate whether exposure to a pulsed high-frequency electromagnetic field (pulsed EMF) emitted by a mobile phone has short-term effects on the inhibitory control of saccades. METHODS: A double-blind, counterbalanced crossover study design was employed. We assessed the performance of 10 normal subjects on antisaccade (AS) and cued saccade (CUED) tasks as well as two types of overlap saccade (OL1, OL2) task before and after 30 min of exposure to EMF emitted by a mobile phone or sham exposure. RESULTS: After EMF or sham exposure, we observed a slight but significant shortening of latency in the CUED and OL2 tasks. AS amplitude decreased as well as the saccade velocities in the AS, CUED, and OL1 tasks after exposure. These changes occurred regardless of whether exposure was real or sham. The frequencies of pro-saccades in the AS task, saccades to cue in the CUED task, and prematurely initiated saccades in the overlap (OL2) task did not change significantly after real or sham EMF exposure. CONCLUSIONS: Thirty minutes of mobile phone exposure has no significant short-term effect on the inhibitory control of saccades. SIGNIFICANCE: The cortical processing responsible for saccade inhibition is not affected by exposure to EMF emitted by a mobile phone.

(E) Panda NK, Jain R, Bakshi J, Munjal S. Audiologic disturbances in long-term mobile phone users. J Otolaryngol Head Neck Surg. 39(1):5-11, 2010. (HU, CE, PE)

INTRODUCTION: There is general concern regarding the possible hazardous health effects of exposure to radiofrequency electromagnetic radiation emitted from mobile phones. This study aimed to assess the effects of chronic exposure to electromagnetic waves emitted from Global System for Mobile Communication (GSM) mobile phones on auditory functions. **MATERIAL AND METHODS:** A retrospective, cross-sectional, randomized, case control study was carried out in a tertiary care hospital. One hundred twelve subjects who were long-term mobile phone users (more than 1 year) and 50 controls who had never used a mobile phone underwent a battery of audiologic investigations including pure-tone audiometry (both speech and high frequency), tympanometry, distortion product otoacoustic emissions, auditory brain responses, and middle latency responses. Changes in the various parameters were studied in the mobile phone- and non-mobile phone-using ears of subjects and corresponding ears of the controls to ascertain the effects of electromagnetic exposure. **RESULTS:** There was no significant difference between users and controls for any of the audiologic parameters. However, trends for audiologic abnormalities were seen within the users. High-frequency loss and absent distortion product otoacoustic emissions were observed with an increase in the duration of mobile phone use, excessive use of mobile phones, and age more than 30 years. Additionally, users with some complaints during mobile phone use demonstrated absent distortion product otoacoustic emissions and abnormalities in auditory brainstem response. **CONCLUSION:** Long-term and intensive mobile phone use may cause inner ear damage. A large sample size would be required to reach definitive conclusions.

(E) Panda NK, Modi R, Munjal S, Virk RS. Auditory changes in mobile users: is evidence forthcoming? Otolaryngol Head Neck Surg. 144(4):581-585, 2011. (HU, CE, PE)

OBJECTIVE: Genuine concerns are being raised as to the potential health risks posed by electromagnetic frequency exposure secondary to mobile phone usage. This study was undertaken to assess and compare potential changes in hearing function at the level of the inner ear and central auditory pathway due to chronic exposure to electromagnetic waves from both global system for mobile communications (GSM) and code division multiple access (CDMA) mobile phone usage. DESIGN: Cohort study. SETTING: Tertiary referral center. SUBJECTS AND METHODS: One hundred twenty-five subjects who were long-term mobile phone users (more than 1 year; 63 GSM and 62 CDMA) and 58 controls who had never used mobile phones underwent audiological investigations including pure tone audiometry (250-12 kHz), tympanometry, distortion product otoacoustic emissions (DPOAE), auditory brain responses (ABR), and middle latency responses (MLRs). The changes in various parameters were studied in mobile-using and non-mobile-using ears of both GSM and CDMA subjects and corresponding ears of the controls to ascertain the effects of electromagnetic exposure. RESULTS: GSM and CDMA users were found to be at a significantly higher risk of having DPOAE absent as compared with controls ($P < .05$). They were found to have higher speech frequency thresholds and lower MLR wave and Na and Pa amplitudes. More than 3 years of mobile phone usage emerged as a risk factor ($P < .05$). The damage done was bilateral, with the quantum of damage being the same for both GSM and CDMA. CONCLUSION: Long-term and intensive GSM and CDMA mobile phone use may cause damage to cochlea as well as the auditory cortex.

(E) Papageorgiou CC, Hountala CD, Maganioti AE, Kyprianou MA, Rabavilas AD, Papadimitriou GN, Capsalis CN. Effects of wi-fi signals on the p300 component of event-related potentials during an auditory hayling task. J Integr Neurosci. 10(2):189-202, 2011 (HU, EE)

The P300 component of event-related potentials (ERPs) is believed to index attention and working memory (WM) operation of the brain. The present study focused on the possible gender-related effects of Wi-Fi (Wireless Fidelity) electromagnetic fields (EMF) on these processes. Fifteen male and fifteen female subjects, matched for age and education level, were investigated while performing a modified version of the Hayling Sentence Completion test adjusted to induce WM. ERPs were recorded at 30 scalp electrodes, both without and with the exposure to a Wi-Fi signal. P300 amplitude values at 18 electrodes were found to be significantly lower in the response inhibition condition than in the response initiation and baseline conditions. Independent of the above effect, within the response inhibition condition there was also a significant gender X radiation interaction effect manifested at 15 leads by decreased P300 amplitudes of males in comparison to female subjects only at the presence of EMF. In conclusion, the present findings suggest that Wi-Fi exposure may exert gender-related alterations on neural activity associated with the amount of attentional resources engaged during a linguistic test adjusted to induce WM.

(NE) Paparini A, Rossi P, Gianfranceschi G, Brugaletta V, Falsaperla R, De Luca P, Romano Spica V. No evidence of major transcriptional changes in the brain of mice exposed to 1800 MHz GSM signal. Bioelectromagnetics. 29(4):312-323, 2008. (AS, CH)

To analyze possible effects of microwaves on gene expression, mice were exposed to global system for mobile communication (GSM) 1800 MHz signal for 1 h at a whole body SAR of 1.1 W/kg. Gene expression was studied in the whole brain, where the average SAR was 0.2 W/kg, by expression microarrays containing over 22,600 probe sets. Comparison of data from sham and exposed animals showed no significant difference in gene expression modulation. However, when less stringent constraints were adopted to analyze microarray results, 75 genes were found to be modulated following exposure. Forty-two probes showed fold changes ranging from 1.5 to 2.8, whereas 33 were down-regulated from 0.67- to 0.29-fold changes, but these differences in gene expression were not confirmed by real-time PCR. Under these specific limited conditions, no consistent indication of gene expression modulation in whole mouse brain was found associated to GSM 1800 MHz exposure.

(E) Parazzini M, Ravazzani P, Tognola G, Thuróczy G, Molnar FB, Sacchettini A, Ardesi G, Mainardi LT. Electromagnetic fields produced by GSM cellular phones and heart rate variability. Bioelectromagnetics. 28(2):122-129, 2007. (HU, PE)

In this study, 26 healthy young volunteers were submitted to 900 MHz (2 W) GSM cellular phone exposure and to sham exposure in separate sessions. The study was designed to assess cardiac regulatory mechanism in different autonomic nervous system (ANS) states during exposure to low-intensity EMF. Rest-to-stand protocol was applied to evaluate ANS in quiet condition (rest, vagal prevalence) and after a sympathetic activation (stand). The procedure is conducted twice in a double-blind design: once with a genuine EMF exposure and once with a sham exposure (at least 24 h apart). During each session three-leads electrocardiograms were recorded and RR series extracted off-line. Time domain and frequency domain HRV parameters were calculated in every phase of the protocol and during different exposures. The analysis of the data show there was no statistically significant effect due to EMF exposure both on main (i.e., RR mean) and most of the other HRV parameters. A weak interaction between some HRV parameters (i.e., SDNN, TINN, and triangular index in time domain and LF power in frequency domain analysis) and RF exposure was observed and this effect seems to be gathered around the sympathetic response to stand.

(NE) Parazzini M, Sibella F, Lutman ME, Mishra S, Moulin A, Sliwinska-Kowalska M, Woznicka E, Politanski P, Zmyslony M, Thuroczy G, Molnár F, Kubinyi G, Tavartkiladze G, Bronyakin S, Uloziene I, Uloza V, Gradauskiene E, Ravazzani P. Effects of UMTS cellular phones on human hearing: results of the European project EMFnEAR. Radiat Res. 172(2):244-251, 2009. (HU, PE)

The European project EMFnEAR was undertaken to assess potential changes in human auditory function after a short-term exposure to radiofrequency (RF) radiation produced by UMTS (Universal Mobile Telecommunication System) mobile phones. Participants were healthy young adults with no hearing or ear disorders. Auditory function was assessed immediately before and after exposure to radiofrequency radiation, and only the exposed ear was tested. Tests for the assessment of auditory function were hearing threshold level (HTL), distortion product otoacoustic emissions (DPOAE), contralateral suppression of transiently evoked otoacoustic emission (CAS effect on TEOAE), and auditory evoked potentials (AEP). The exposure consisted of speech at a typical conversational level delivered via an earphone to one ear, plus genuine or sham RF-radiation exposure produced by a commercial phone controlled by a personal computer. Results from 134 participants did not show any consistent pattern of effects on the auditory system after a 20-min UMTS exposure at the maximum output of the phone with 69 mW/kg SAR in the cochlea region in a double blind comparison of genuine and sham exposure. An isolated effect on the hearing threshold at high frequencies was identified, but this was statistically nonsignificant after correction for multiple comparisons. It is concluded that UMTS short-term exposure at the maximum output of consumer mobile phones does not cause measurable immediate effects on the human auditory system.

(E) Partsvania B, Sulaberidze T, Shoshiashvili L, Modebadze Z. Acute effect of exposure of mollusk single neuron to 900-MHz mobile phone radiation. Electromagn Biol Med. 30(3):170-179, 2011. (CS, EE)

The goal of the present work was to explore the influence of commercially available cell phone irradiation on the single neuron excitability and memory processes. A Transverse Electromagnetic Cell (TEM Cell) was used to expose single neurons of mollusk to the electromagnetic field. Finite-Difference Time-Domain (FDTD) method was used for modeling the TEM Cell and the electromagnetic field interactions with living nerve ganglion and neurons. Neuron electrophysiology was investigated using standard microelectrode technique. The specific absorption rate (SAR) deposited into the single neuron was calculated to be 0.63 W/kg with a temperature increment of 0.1°C. After acute exposure, average firing threshold of the action potentials was not changed. However, the average latent period was significantly decreased. This indicates that together with latent period the threshold and the time of habituation might be altered during exposure. However, these alterations are transient and only latent period remains on the changed level.

(E) Pelletier A, Delanaud S, Décima P, Thuroczy G, de Seze R, Cerri M, Bach V, Libert JP, Loos N. Effects of chronic exposure to radiofrequency electromagnetic fields on energy balance in developing rats. Environ Sci Pollut Res Int. 2012 Nov 10. [Epub ahead of print] (AS, LI, CE, BE, PE, SL)

The effects of radiofrequency electromagnetic fields (RF-EMF) on the control of body energy balance in developing organisms have not been studied, despite the involvement of energy status in vital physiological functions. We examined the effects of chronic RF-EMF exposure (900 MHz, 1 V m(-1)) on the main functions involved in body energy homeostasis (feeding behaviour, sleep and thermoregulatory processes). Thirteen juvenile male Wistar rats were exposed to continuous RF-EMF for 5 weeks at 24 °C of air temperature (T (a)) and compared with 11 non-exposed animals. Hence, at the beginning of the 6th week of exposure, the functions were recorded at T (a) of 24 °C and then at 31 °C. We showed that the frequency of rapid eye movement sleep episodes was greater in the RF-EMF-exposed group, independently of T (a) (+42.1 % at 24 °C and +31.6 % at 31 °C). The other effects of RF-EMF exposure on several sleep parameters were dependent on T (a). At 31 °C, RF-EMF-exposed animals had a significantly lower subcutaneous tail temperature (-1.21 °C) than controls at all sleep stages; this suggested peripheral vasoconstriction, which was confirmed in an experiment with the vasodilatator prazosin. Exposure to RF-EMF also increased daytime food intake (+0.22 g h(-1)). Most of the observed effects of RF-EMF exposure were dependent on T (a). Exposure to RF-EMF appears to modify the functioning of vasomotor tone by acting peripherally through α-adrenoceptors. The elicited vasoconstriction may restrict body cooling, whereas energy intake increases. Our results show that RF-EMF exposure can induce energy-saving processes without strongly disturbing the overall sleep pattern.

(NE) Perentos N, Croft RJ, McKenzie RJ, Cvetkovic D, Cosic I. Comparison of the effects of continuous and pulsed mobile phone like RF exposure on the human EEG. Australas Phys Eng Sci Med. 30(4):274-280, 2007. (HU, EE)

It is not clear yet whether Global System for Mobiles (GSM) mobile phone radiation has the ability to interfere with normal resting brain function. There have been reports that GSM exposure increases alpha band power, and does so only when the signal is modulated at low frequencies (Huber, R., Treyer, V., Borbely, A. A., Schuderer, J., Gottselig, J. M., Landolt, H.P., Werth, E., Berthold,T., Kuster, N., Buck, A and Achermann, P. Electromagnetic fields, such as those from mobile phones, alter regional cerebral blood flow and sleep and waking EEG. J Sleep Res 11, 289-295, 2002.) However, as that research employed exposure distributions that are not typical of normal GSM handset usage (deep brain areas were overexposed), it remains to be determined whether a similar result patterning would arise from a more representative exposure. In this fully counterbalanced cross-over design, we recruited 12 participants and tried to replicate the modulation linked post exposure alpha band power increase described above, but with an exposure source (dipole antenna) more closely resembling that of a real GSM handset. Exposures lasted for 15 minutes. No changes to alpha power were found for either modulated or unmodulated radiofrequency fields, and thus we failed to replicate the above results. Possible reasons for this failure to replicate are discussed, with the main reason argued to be the lower and more representative exposure distribution employed in the present study. In addition we investigated the possible GSM exposure related effects on the non-linear features of the resting electroencephalogram using the Approximate Entropy (ApEn) method of analysis. Again, no effect was demonstrated for either modulated or unmodulated radiofrequency exposures.

(NE) Platano D, Mesirca P, Paffi A, Pellegrino M, Liberti M, Apollonio F, Bersani F, Aicardi G. Acute exposure to low-level CW and GSM-modulated 900 MHz radiofrequency does not affect Ba 2+ currents through voltage-gated calcium channels in rat cortical neurons. Bioelectromagnetics. 28(8):599-607, 2007. (CS, EE)

We have studied the non-thermal effects of radiofrequency (RF) electromagnetic fields (EMFs) on Ba(2+) currents (I Ba 2+) through voltage-gated calcium channels (VGCC), recorded in primary cultures of rat cortical neurons using the patch-clamp technique. To assess whether low-level acute RF field exposure could modify the amplitude and/or the voltage-dependence of

I Ba 2+, Petri dishes containing cultured neurons were exposed for 1-3 periods of 90 s to 900 MHz RF-EMF continuous wave (CW) or amplitude-modulated according to global system mobile communication standard (GSM) during whole-cell recording. The specific absorption rates (SARs) were 2 W/kg for CW and 2 W/kg (time average value) for GSM-modulated signals, respectively. The results obtained indicate that single or multiple acute exposures to either CW or GSM-modulated 900 MHz RF-EMFs do not significantly alter the current amplitude or the current-voltage relationship of I Ba 2+, through VGCC.

(NE) Poulletier de Gannes F, Haro E, Hurtier A, Taxile M, Ruffié G, Billaudel B, Veyret B, Lagroye I. Effect of exposure to the edge signal on oxidative stress in brain cell models. Radiat Res. 175(2):225-230, 2011. (CS, OX)

In this study we investigated the effect of the Enhanced Data rate for GSM Evolution (EDGE) signal on cells of three human brain cell lines, SH-SY5Y, U87 and CHME5, used as models of neurons, astrocytes and microglia, respectively, as well as on primary cortical neuron cultures. SXC-1800 waveguides (IT'IS-Foundation, Zürich, Switzerland) were modified for in vitro exposure to the EDGE signal radiofrequency (RF) radiation at 1800 MHz. Four exposure conditions were tested: 2 and 10 W/kg for 1 and 24 h. The production of reactive oxygen species (ROS) was measured by flow cytometry using the dichlorofluorescein diacetate (DCFH-DA) probe at the end of the 24-h exposure or 24 h after the 1-h exposure. Rotenone treatment was used as a positive control. All cells tested responded to rotenone treatment by increasing ROS production. These findings indicate that exposure to the EDGE signal does not induce oxidative stress under these test conditions, including 10 W/kg. Our results are in agreement with earlier findings that RF radiation alone does not increase ROS production.

(NE) Prochnow N, Gebing T, Ladage K, Krause-Finkeldey D, El Ouardi A, Bitz A, Streckert J, Hansen V, Dermietzel R. Electromagnetic field effect or simply stress? Effects of UMTS exposure on hippocampal longterm plasticity in the context of procedure related hormone release. PLoS One. 6(5):e19437, 2011. (AS, EE)

Harmful effects of electromagnetic fields (EMF) on cognitive and behavioural features of humans and rodents have been controversially discussed and raised persistent concern about adverse effects of EMF on general brain functions. In the present study we applied radio-frequency (RF) signals of the Universal Mobile Telecommunications System (UMTS) to full brain exposed male Wistar rats in order to elaborate putative influences on stress hormone release (corticosteron; CORT and adrenocorticotropic hormone; ACTH) and on hippocampal derived synaptic long-term plasticity (LTP) and depression (LTD) as electrophysiological hallmarks for memory storage and memory consolidation. Exposure was computer controlled providing blind conditions. Nominal brain-averaged specific absorption rates (SAR) as a measure of applied mass-related dissipated RF power were 0, 2, and 10 W/kg over a period of 120 min. Comparison of cage exposed animals revealed, regardless of EMF exposure, significantly increased CORT and ACTH levels which corresponded with generally decreased field potential slopes and amplitudes in hippocampal LTP and LTD. Animals following SAR exposure of 2 W/kg (averaged over the whole brain of 2.3 g tissue mass) did not differ from the sham-exposed group in LTP and LTD experiments. In contrast, a significant reduction in LTP and LTD was observed at the high power rate of SAR (10 W/kg). The results demonstrate that a rate of 2 W/kg displays no adverse impact on LTP and LTD, while 10 W/kg leads to significant effects on the electrophysiological parameters, which can be clearly distinguished from the stress derived background. Our findings suggest that UMTS exposure with SAR in the range of 2 W/kg is not harmful to critical markers for memory storage and memory consolidation, however, an influence of UMTS at high energy absorption rates (10 W/kg) cannot be excluded.

(E) Qin F, Yuan H, Nie J, Cao Y, Tong J. [Effects of nano-selenium on cognition performance of mice exposed in 1800 MHz radiofrequency fields]. Wei Sheng Yan Jiu. 43(1):16-21, 2014. [Article in Chinese] (AS, CE, BE, CH, OX)

OBJECTIVE: To study the effects of nano-selenium (NSe) on cognition performance of mice exposed to 1800 MHz radiofrequency fields (RF).METHODS: Male mice were randomly divided into four groups, control and nano-Se low, middle and high dose groups (L, M, H). Each group was sub-divided into three groups, RF 0 min, RF 30 min and RF 120 min. Nano-se solution (2, 4 and 8 microg/ml) were administered to mice of L, M, H groups by intra-gastric injection respectively, 0.5 ml/d for 50 days, the control group was administered with distilled water. At the 21st day, the mice in RF subgroup were exposed to 208 microW/cm2 1800 MHz radiofrequency fields (0, 30 and 120 min/d respectively) for 30 days. The cognitive ability of the mice were tested with Y-maze. Further, the levels of MDA, GABA, Glu, Ach and the activities of CAT and GSH-Px in cerebra were measured. RESULTS: Significant impairments in learning and memory ($P < 0.05$) were observed in the RF 120 min group, and with reduction of the Ach level and the activities of CAT and GSH-Px and increase of the content of GABA, Glu and MDA in cerebrum. NSe enhanced cognitive performance of RF mice, decreased GABA, Glu and MDA levels, increased Ach levels, GSH-Px and CAT activities. CONCLUSION: NSe could improve cognitive impairments of mice exposed to RF, the mechanism of which might involve the increasing antioxidation, decreasing free radical content and the changes of cerebra neurotransmitters.

(NE) Rağbetli MC, Aydinlioğlu A, Koyun N, Rağbetli C, Karayel M. Effect of prenatal exposure to mobile phone on pyramidal cell numbers in the mouse hippocampus: a stereological study. Int J Neurosci. 119(7):1031-1041, 2009. (AS, ME, DE)

Because of the possible risk factor for the health, World Health Organization (WHO) recommended the study with animals on the developing nervous system concerning the exposure to radiofrequency (RF) field. A few studies related to hippocampal exposure are available, which indicate the impact of RF field in some parameters. The present study investigated the effect of exposure to mobile phone on developing hippocampus. Male and female Swiss albino mice were housed as control and mobile phone exposed groups. The pregnant animals in tested group were exposed to the effects of mobile phone in a room possessing the exposure system. The left hemispheres of the brains were processed by frozen microtome. The sections obtained were stained with Hematoxylin & Eosin. For cell counting by the optical fractionator method, a pilot study was first performed. Hippocampal areas were analyzed using Axiovision software running on a personal computer. The optical dissector, systematically and randomly spaced, was focused to the widest profile of the pyramidal cell nucleus. No significant difference in pyramidal cell number of total Cornu Ammonis (CA) sectors of hippocampus was found between the control and the mobile phone exposed groups ($p > .05$). It was concluded that further study is needed in this field due to popular use of mobile telephones and relatively high exposure to the developing brain.

(E) Rağbetli MC, Aydinlioğlu A, Koyun N, Rağbetli C, Bektas S, Ozdemir S. The effect of mobile phone on the number of Purkinje cells: a stereological study. Int J Radiat Biol. 86(7):548-554, 2010. (AS, ME, DE)

PURPOSE: The World Health Organisation proposed an investigation concerning the exposure of animals to radiofrequency fields because of the possible risk factor for health. At power frequencies there is evidence to associate both childhood leukaemia and brain tumours with magnetic field exposures. There is also evidence of the effect of mobile phone exposure on both cognitive functions and the cerebellum. Purkinje cells of the cerebellum are also sensitive to high dose microwave exposure in rats. The present study investigated the effect of exposure to mobile phone on the number of Purkinje and granule neurons in the developing cerebellum. **MATERIAL AND METHODS:** Male and female Swiss albino mice were housed as control and mobile phone-exposed groups. Pregnant animals in the experimental group were exposed to Global System for Mobile Communication (GSM) mobile phone radiation at 890-915 MHz at 0.95 W/Kg specific absorption rate (SAR). The cerebella were processed by frozen microtome. The sections obtained were stained with Haematoxylin-eosin and cresyl violet. For cell counting by the optical fractionator method, a pilot study was firstly performed. Cerebellar areas were analysed by using Axiovision software running on a personal computer. The optical dissectors were systematically spaced at random, and focused to the widest profile of the neuron cell nucleus. **RESULTS:** A significant decrease in the number of Purkinje cells and a tendency for granule cells to increase in cerebellum was found. **CONCLUSION:** Further studies in this area are needed due to the popular use of mobile telephones and relatively high exposure on developing brain.

(E) Razavinasab M, Moazzami K, Shabani M. Maternal mobile phone exposure alters intrinsic electrophysiological properties of CA1 pyramidal neurons in rat offspring. Toxicol Ind Health. 2014 Mar 6. [Epub ahead of print] (AS, CE, BE, DE, EE)

Some studies have shown that exposure to electromagnetic field (EMF) may result in structural damage to neurons. In this study, we have elucidated the alteration in the hippocampal function of offspring Wistar rats (n = 8 rats in each group) that were chronically exposed to mobile phones during their gestational period by applying behavioral, histological, and electrophysiological tests. Rats in the EMF group were exposed to 900 MHz pulsed-EMF irradiation for 6 h/day. Whole cell recordings in hippocampal pyramidal cells in the mobile phone groups did show a decrease in neuronal excitability. Mobile phone exposure was mostly associated with a decrease in the number of action potentials fired in spontaneous activity and in response to current injection in both male and female groups. There was an increase in the amplitude of the afterhyperpolarization (AHP) in mobile phone rats compared with the control. The results of the passive avoidance and Morris water maze assessment of learning and memory performance showed that phone exposure significantly altered learning acquisition and memory retention in male and female rats compared with the control rats. Light microscopy study of brain sections of the control and mobile phone-exposed rats showed normal morphology. Our results suggest that exposure to mobile phones adversely affects the cognitive performance of both female and male offspring rats using behavioral and electrophysiological techniques.

(E) Redmayne M, Smith E, and Abramson MJ. The relationship between adolescents' well-being and their wireless phone use: a cross-sectional study. Environmental Health 12(1):90, 2013. (HU, BE)

Background. The exposure of young people to radiofrequency electromagnetic fields (RF-EMFs) has increased rapidly in recent years with their increased use of cellphones and use of cordless phones and WiFi. We sought to ascertain associations between New Zealand early-adolescents' subjective well-being and self-reported use of, or exposure to, wireless telephone and internet technology. Methods. In this cross-sectional survey, participants completed questionnaires in class about their cellphone and cordless phone use, their self-reported well-being, and possible confounding information such as whether they had had influenza recently or had a television in the bedroom. Parental questionnaires provided data on whether they had WiFi at home and cordless phone ownership and model. Data were analysed with Ordinal Logistic Regression adjusting for common confounders. Odds ratios (OR) and 95% confidence intervals were calculated. Results. The number and duration of cellphone and cordless phone calls were associated with increased risk of headaches (>6 cellphone calls over 10 minutes weekly, adjusted OR 2.4, CI 1.2-4.8; >15 minutes cordless use daily adjusted OR 1.74, CI 1.1-2.9)). Texting and extended use of wireless phones was related to having a painful 'texting' thumb). Using a wired cellphone headset was associated with tinnitus (adjusted OR 1.8, CI 1.0-3.3), while wireless headsets were associated with headache (adjusted OR 2.2, CI 1.1-4.5), feeling down/depressed (adjusted OR 2.0, CI 1.1-3.8), and waking in the night (adjusted OR 2.4, CI 1.2-4.8). Several cordless phone frequencies bands were related to tinnitus, feeling down/depressed and sleepiness at school, while the last of these was also related to modulation. Waking nightly was less likely for those with WiFi at home (adjusted OR 0.7, CI 0.4-0.99). Being woken at night by a cellphone was strongly related to tiredness at school (OR 4.1, CI 2.2-7.7). Conclusions . There were more statistically significant associations (36%) than could be expected by chance (5%). Several were dose-dependent relationships. To safeguard young people's well-being, we suggest limiting their use of cellphones and cordless phones to less than 15 minutes daily, and employing a speaker-phone device for longer daily use. We recommend parental measures are taken to prevent young people being woken by their cellphones.

(E) Regel SJ, Tinguely G, Schuderer J, Adam M, Kuster N, Landolt HP, Achermann P. Pulsed radio-frequency electromagnetic fields: dose-dependent effects on sleep, the sleep EEG and cognitive performance. J Sleep Res. 16(3):253-258, 2007. (HU, EE, BE, SL)

To establish a dose-response relationship between the strength of electromagnetic fields (EMF) and previously reported effects on the brain, we investigated the influence of EMF exposure by varying the signal intensity in three experimental sessions. The head of 15 healthy male subjects was unilaterally exposed for 30 min prior to sleep to a pulse-modulated EMF (GSM handset like signal) with a 10 g-averaged peak spatial specific absorption rate of (1) 0.2 W kg(-1), (2) 5 W kg(-1), or (3) sham exposed in a double-blind, crossover design. During exposure, subjects performed two series of three computerized cognitive tasks, each presented in a fixed order [simple reaction time task, two-choice reaction time task (CRT), 1-, 2-, 3-back task]. Immediately after exposure, night-time sleep was polysomnographically recorded for 8 h. Sleep architecture was not affected by EMF exposure. Analysis of the sleep electroencephalogram (EEG) revealed a dose-dependent increase of power in the spindle frequency range in non-REM sleep. Reaction speed decelerated with increasing field intensity in the 1-back task, while accuracy in the CRT and N-back task were not affected in a dose-dependent manner. In summary, this study reveals first indications of a dose-response relationship between EMF field intensity and its effects on brain physiology as demonstrated by changes in the sleep EEG and in cognitive performance.

(NE) Riddervold IS, Pedersen GF, Andersen NT, Pedersen AD, Andersen JB, Zachariae R, Mølhave L, Sigsgaard T, Kjaergaard SK. Cognitive function and symptoms in adults and adolescents in relation to rf radiation from UMTS base stations. Bioelectromagnetics. 29(4):257-267, 2008. (HU, BE)

There is widespread public concern about the potential adverse health effects of mobile phones in general and their associated base stations in particular. This study was designed to investigate the acute effects of radio frequency (RF) electromagnetic fields (EMF) emitted by the Universal Mobile Telecommunication System (UMTS) mobile phone base stations on human cognitive function and symptoms. Forty adolescents (15-16 years) and 40 adults (25-40 years) were exposed to four conditions: (1) sham, (2) a Continuous Wave (CW) at 2140 MHz, (3) a signal at 2140 MHz modulated as UMTS and (4) UMTS at 2140 MHz including all control features in a randomized, double blinded cross-over design. Each exposure lasted 45 min. During exposure the participants performed different cognitive tasks with the Trail Making B (TMB) test as the main outcome and completed a questionnaire measuring self reported subjective symptoms. No statistically significant differences between the UMTS and sham conditions were found for performance on TMB. For the adults, the estimated difference between UMTS and sham was -3.2% (-9.2%; 2.9%) and for the adolescents 5.5% (-1.1%; 12.2%). No significant changes were found in any of the cognitive tasks. An increase in 'headache rating' was observed when data from the adolescents and adults were combined (P = 0.027), an effect that may be due to differences at baseline. In conclusion, the primary hypothesis that UMTS radiation reduces general performance in the TMB test was not confirmed. However, we suggest that the hypothesis of subjective symptoms and EMF exposure needs further research.

(NE) Sakurai T, Kiyokawa T, Narita E, Suzuki Y, Taki M, Miyakoshi J. Analysis of gene expression in a human-derived glial cell line exposed to 2.45 GHz continuous radiofrequency electromagnetic fields. J Radiat Res. 52(2):185-192, 2011. (CS, CH)

The increasing use of mobile phones has aroused public concern regarding the potential health risks of radiofrequency (RF) fields. We investigated the effects of exposure to RF fields (2.45 GHz, continuous wave) at specific absorption rate (SAR) of 1, 5, and 10 W/kg for 1, 4, and 24 h on gene expression in a normal human glial cell line, SVGp12, using DNA microarray. Microarray analysis revealed 23 assigned gene spots and 5 non-assigned gene spots as prospective altered gene spots. Twenty-two genes out of the 23 assigned gene spots were further analyzed by reverse transcription-polymerase chain reaction to validate the results of microarray, and no significant alterations in gene expression were observed. Under the experimental conditions used in this study, we found no evidence that exposure to RF fields affected gene expression in SVGp12 cells.

(E) Sarapultseva EI, Igolkina JV, Tikhonov VN, Dubrova YE.THE IN VIVO EFFECTS OF LOW-INTENSITY RADIOFREQUENCY FIELDS ON THE MOTOR ACTIVITY OF PROTOZOA. Int J Radiat Biol. 2013 Nov 25. [Epub ahead of print] (AS, BE, LI)
Purpose: To analyze the direct and transgenerational effects of exposure to low-dose 1 GHz (mobile phone/wireless telecommunication range) and 10 GHz (radar/satellite communication range) radiofrequency electromagnetic fields (RF-EMF) on the motility of ciliates Spirostomum ambiguum. Materials and Methods: S. ambiguum were exposed to 1 GHz and 10 GHz RF-EMF with power flux densities (PD) ranging from 0.05 to 0.5 W/m^2 over a period of time from 0.05 to 10 h. The motility of directly exposed ciliates and their non-exposed progeny across 10-15 generations was measured. Results: Exposure to 0.1 W/m^2 of either 1 or 10 GHz RF-EMF resulted in a significant decrease in the motility. The dose of exposure capable of altering the mobility of ciliates was inversely correlated with the flux density of RF-EMF. The motility of the non-exposed progeny of ciliates irradiated with 0.1 W/m^2 of 10 GHz RF-EMF remained significantly compromised, at least, across 10-15 generations, thus indicating the presence of transgenerational effects. Conclusions: The results of our study show that low-dose exposure to RF-EMF can significantly affect the motility of irradiated ciliates and their non-exposed offspring, thus providing further insights into the unknown mechanisms underlying the in vivo effects of RF-EMF.

(NE) Sauter C, Dorn H, Bahr A, Hansen ML, Peter A, Bajbouj M, Danker-Hopfe H. Effects of exposure to electromagnetic fields emitted by GSM 900 and WCDMA mobile phones on cognitive function in young male subjects. Bioelectromagnetics. 32(3):179-190, 2011. (HU, BE)

Results of studies on the possible effects of electromagnetic fields emitted by mobile phones on cognitive functions are contradictory, therefore, possible effects of long-term (7 h 15 min) electromagnetic field (EMF) exposure to handset-like signals of Global System for Mobile Communications (GSM) 900 and Wideband Code-Division Multiple Access (WCDMA) on attention and working memory were studied. The sample comprised 30 healthy male subjects (mean ± SD: 25.3 ± 2.6 years), who were tested on nine study days in which they were exposed to three exposure conditions (sham, GSM 900 and WCDMA) in a randomly assigned and balanced order. All tests were presented twice (morning and afternoon) on each study day within a fixed timeframe. Univariate comparisons revealed significant changes when subjects were exposed to GSM 900 compared to sham, only in the vigilance test. In the WCDMA exposure condition, one parameter in the vigilance and one in the test on divided attention were altered compared to sham. Performance in the selective attention test and the n-back task was not affected by GSM 900 or WCDMA exposure. Time-of-day effects were evident for the tests on divided and selective attention, as well as for working memory. After correction for multiple testing, only time-of-day effects remained significant in two tests, resulting in faster reactions in the afternoon trials. The results of the present study do not provide any evidence of an EMF effect on human cognition, but they underline the necessity to control for time of day.

(E) Schmid MR, Loughran SP, Regel SJ, Murbach M, Bratic Grunauer A, Rusterholz T, Bersagliere A, Kuster N, Achermann P. Sleep EEG alterations: effects of different pulse-modulated radio frequency electromagnetic fields. J Sleep Res. 21(1):50-58, 2012a. (HU, EE, BE, SL, WS)

Previous studies have observed increases in electroencephalographic power during sleep in the spindle frequency range (approximately 11-15 Hz) after exposure to mobile phone-like radio frequency electromagnetic fields (RF EMF). Results also suggest that pulse modulation of the signal is crucial to induce these effects. Nevertheless, it remains unclear which specific elements of the field are responsible for the observed changes. We investigated whether pulse-modulation frequency components in the range of sleep spindles may be involved in mediating these effects. Thirty young healthy men were exposed, at weekly intervals, to three different conditions for 30 min directly prior to an 8-h sleep period. Exposure consisted of a 900-MHz RF EMF, pulse modulated at 14 Hz or 217 Hz, and a sham control condition. Both active conditions had a peak spatial specific absorption rate of 2 W kg(-1) . During exposure subjects performed three different cognitive tasks (measuring attention, reaction speed and working memory), which were presented in a fixed order. Electroencephalographic power in the spindle frequency range was increased during non-rapid eye movement sleep (2nd episode) following the 14-Hz pulse-modulated condition. A similar but non-significant increase was also observed following the 217-Hz pulse-modulated condition. Importantly, this exposure-induced effect showed considerable individual variability. Regarding cognitive performance, no clear exposure-related effects were seen. Consistent with previous findings, our results provide further evidence that pulse-modulated RF EMF alter brain physiology, although the time-course of the effect remains variable across studies. Additionally, we demonstrated that modulation frequency components within a physiological range may be sufficient to induce these effects.

(E) Schmid MR, Murbach M, Lustenberger C, Maire M, Kuster N, Achermann P, Loughran SP. Sleep EEG alterations: effects of pulsed magnetic fields versus pulse-modulated radio frequency electromagnetic fields. J Sleep Res. 21(6):620-629, 2012b. (HU, EE, SL)

Studies have repeatedly shown that electroencephalographic power during sleep is enhanced in the spindle frequency range following radio frequency electromagnetic field exposures pulse-modulated with fundamental frequency components of 2, 8, 14 or 217 Hz and combinations of these. However, signals used in previous studies also had significant harmonic components above 20 Hz. The current study aimed: (i) to determine if modulation components above 20 Hz, in combination with radio frequency, are necessary to alter the electroencephalogram; and (ii) to test the demodulation hypothesis, if the same effects occur after magnetic field exposure with the same pulse sequence used in the pulse-modulated radio frequency exposure. In a randomized double-blind crossover design, 25 young healthy men were exposed at weekly intervals to three different conditions for 30 min before sleep. Cognitive tasks were also performed during exposure. The conditions were a 2-Hz pulse-modulated radio frequency field, a 2-Hz pulsed magnetic field, and sham. Radio frequency exposure increased electroencephalogram power in the spindle frequency range. Furthermore, delta and theta activity (non-rapid eye movement sleep), and alpha and delta activity (rapid eye movement sleep) were affected following both exposure conditions. No effect on sleep architecture and no clear impact of exposure on cognition was observed. These results demonstrate that both pulse-modulated radio frequency and pulsed magnetic fields affect brain physiology, and the presence of significant frequency components above 20 Hz are not fundamental for these effects to occur. Because responses were not identical for all exposures, the study does not support the hypothesis that effects of radio frequency exposure are based on demodulation of the signal only.

(E) Sharma A, Sisodia R, Bhatnagar D, Saxena VK. Spatial memory **and learning performance and its relationship to protein synthesis of Swiss albino mice exposed to 10 GHz microwaves. Int J Radiat Biol. 2013 Aug 19. [Epub ahead of print] (AS, CE, BE, CH)**

Purpose: To study the possible role of microwave (MW) exposure on spatial memory of Swiss albino mice and its relationship to protein concentration in whole brain. Materials and methods: Mice were exposed to 10 GHz (Giga Hertz) microwaves with the power density of 0.25 mW/cm^2 (milliwatt per centimeter square) with average whole body specific absorption rate (SAR) 0.1790 W/kg daily for 2 hours per day (h/day) for 30 days. After exposure mice were tested for spatial memory performance using Morris water maze test (MWT). For this purpose mice (6-8 weeks old) were divided into two groups (i) sham exposed and, (ii) microwaves exposed. After initial training for two days, MWT was performed for another 6 days. Protein was estimated 48 hours after exposure and immediately after completion of MWT. Results: Both sham exposed and microwave exposed animals showed a significant decrease in escape time with training. Microwave exposed animals had statistically significant higher mean latency to reach the target quadrant compared to sham exposed. A concurrent decrease in protein levels was estimated in whole brain of the exposed mice compared to sham exposed mice. Conclusions: It can be concluded from the current study that exposure to microwave radiation caused decrements in the ability of mice to learn the special memory task, this may be due to simultaneous decrease in protein levels in the brain of mice.

(E) Sirav B, Seyhan N. Effects of radiofrequency radiation exposure on blood-brain barrier permeability in male and female rats. Electromagn Biol Med. 30(4):253-260, 2011. (AS, ME)

During the last several decades, numerous studies have been performed aiming at the question of whether or not exposure to radiofrequency radiation (RFR) influences the permeability of the blood-brain barrier (BBB). The objective of this study was to investigate the effect of RFR on the permeability of BBB in male and female Wistar albino rats. Right brain, left brain, cerebellum, and total brain were analyzed separately in the study. Rats were exposed to 0.9 and 1.8 GHz continuous-wave (CW) RFR for 20 min (at SARs of 4.26 mW/kg and 1.46 mW/kg, respectively) while under anesthesia. Control rats were sham-exposed. Disruption of BBB integrity was detected spectrophotometrically using the Evans-blue dye, which has been used as a BBB tracer and is known to be bound to serum albumin. Right brain, left brain, cerebellum, and total brain were evaluated for BBB permeability. In female rats, no albumin extravasation was found in in the brain after RFR exposure. A significant increase in albumin was found in the brains of the RF-exposed male rats when compared to sham-exposed male brains. These results suggest that exposure to 0.9 and 1.8 GHz CW RFR at levels below the international limits can affect the vascular permeability in the brain of male rats. The possible risk of RFR exposure in humans is a major concern for the society. Thus, this topic should be investigated more thoroughly in the future.

(E) Söderqvist F, Carlberg M, Hardell L. Mobile and cordless telephones, serum transthyretin and the blood-cerebrospinal fluid barrier: a cross-sectional study. Environ Health. 21; 8:19, 2009. (HU, PE)

BACKGROUND: Whether low-intensity radiofrequency radiation damages the blood-brain barrier has long been debated, but little or no consideration has been given to the blood-cerebrospinal fluid barrier. In this cross-sectional study we tested whether long-term and/or short-term use of wireless telephones was associated with changes in the serum transthyretin level, indicating altered transthyretin concentration in the cerebrospinal fluid, possibly reflecting an effect of radiation. **METHODS:** One thousand subjects, 500 of each sex aged 18-65 years, were randomly recruited using the population registry. Data on wireless telephone use were assessed by a postal questionnaire and blood samples were analyzed for serum transthyretin concentrations determined by standard immunonephelometric techniques on a BN Prospec instrument. **RESULTS:** The response rate was 31.4%. Logistic regression of dichotomized TTR serum levels with a cut-point of 0.31 g/l on wireless telephone use yielded increased odds ratios that were statistically not significant. Linear regression of time since first use overall and on the day that blood was withdrawn gave different results for males and females: for men significantly higher serum concentrations of TTR were seen the longer an analogue telephone or a mobile and cordless desktop telephone combined had been used, and in contrast, significantly lower serum levels were seen the longer an UMTS telephone had been used. Adjustment for fractions of use of the different telephone types did not modify the effect for cumulative use or years since first use for mobile telephone and DECT, combined. For women, linear regression gave a significant association for short-term use of mobile and cordless telephones combined, indicating that the sooner blood was withdrawn after the most recent telephone call, the higher the expected transthyretin concentration. **CONCLUSION:** In this hypothesis-generating descriptive study time since first use of mobile telephones and DECT combined was significantly associated with higher TTR levels regardless of how much each telephone type had been used. Regarding short-term use, significantly higher TTR concentrations were seen in women the sooner blood was withdrawn after the most recent telephone call on that day.

(E) Söderqvist F, Carlberg M, Hansson Mild K, Hardell L. Exposure to an 890-MHz mobile phone-like signal and serum levels of S100B and transthyretin in volunteers. Toxicol Lett. 189(1):63-66, 2009. (HU, PE)

Whether low-intensity non-thermal microwave radiation alters the integrity of the blood-brain barrier has been debated since the late 1970s, yet no experimental study has been carried out on humans. The aim of this study was to test, using peripheral markers, whether exposure to a mobile phone-like signal alters the integrity of the human blood-brain and blood-cerebrospinal fluid barriers. A provocation study was carried out that exposed 41 volunteers to a 30 min GSM 890 MHz signal with an average specific energy absorption rate distribution of 1.0 W/kg in the temporal area of the head as measured over any 1g of contiguous tissue. The outcome was assessed by changes in serum concentrations of two putative markers of brain barrier integrity, S100B and transthyretin. Repeated blood sampling before and after the provocation showed no statistically significant increase in the serum levels of S100B, while for transthyretin a statistically significant increase was seen in the final blood sample 60 min after the end of the provocation as compared to the prior sample taken immediately after provocation ($p=0.02$). The clinical significance of this finding, if any, is unknown. Further randomized studies with use of additional more brain specific markers are needed.

(NE) Söderqvist F, Carlberg M, Hardell L. Use of wireless telephones and serum S100B levels: a descriptive cross-sectional study among healthy Swedish adults aged 18-65 years. Sci Total Environ. 407(2):798-805, 2009. (HU, PE)

BACKGROUND: Since the late 1970s, experimental animal studies have been carried out on the possible effects of low-intensive radiofrequency fields on the blood-brain barrier (BBB), but no epidemiological study has been published to date. **OBJECTIVE:** Using serum S100B as a putative marker of BBB dysfunction we performed a descriptive cross-sectional study to investigate whether protein levels were higher among frequent than non-frequent users of mobile and cordless desktop phones. **METHOD:** One thousand subjects, 500 of each sex aged 18-65 years, were randomly recruited using the population registry. Data on wireless phone use were assessed by a postal questionnaire and blood samples were analyzed for S100B. **RESULTS:** The response rate was 31.4%. The results from logistic and linear regression analyses were statistically insignificant, with one exception: the linear regression analysis of latency for UMTS use, which after stratifying on gender remained significant only for men (p = 0.01; n = 31). A low p-value (0.052) was obtained for use of cordless phone (n = 98) prior to giving the blood samples indicating a weak negative association. Total use of mobile and cordless phones over time yielded odds ratio (OR) 0.8 and 95% confidence interval (CI) 0.3-2.0 and use on the same day as giving blood yielded OR=1.1, CI=0.4-2.8. **CONCLUSIONS:** This study failed to show that long- or short-term use of wireless telephones was associated with elevated levels of serum S100B as a marker of BBB integrity. The finding regarding latency of UMTS use may be interesting but it is based on small numbers. Generally, S100B levels were low and to determine whether this association - if causal - is clinically relevant, larger studies with sufficient follow-up are needed.

(E) Söderqvist F, Hardell L, Carlberg M, Mild KH. Radiofrequency fields, transthyretin, and Alzheimer's disease. J Alzheimers Dis. 20(2):599-606, 2010. (HU, PE, MA)

Radiofrequency field (RF) exposure provided cognitive benefits in an animal study. In Alzheimer's disease (AD) mice, exposure reduced brain amyloid-beta (Abeta) deposition through decreased aggregation of Abeta and increase in soluble Abeta levels. Based on our studies on humans on RF from wireless phones, we propose that transthyretin (TTR) might explain the findings. In a cross-sectional study on 313 subjects, we used serum TTR as a marker of cerebrospinal fluid TTR. We found a statistically significantly positive beta coefficient for TTR for time since first use of mobile phones and desktop cordless phones combined (P=0.03). The electromagnetic field parameters were similar for the phone types. In a provocation study on 41 persons exposed for 30 min to an 890-MHz GSM signal with specific absorption rate of 1.0 Watt/kg to the temporal area of the brain, we found statistically significantly increased serum TTR 60 min after exposure. In our cross-sectional study, use of oral snuff also yielded statistically significantly increased serum TTR concentrations and nicotine has been associated with decreased risk for AD and to upregulate the TTR gene in choroid plexus but not in the liver, another source of serum TTR. TTR sequesters Abeta, thereby preventing the formation of Abeta plaques in the brain. Studies have shown that patients with AD have lowered TTR concentrations in the cerebrospinal fluid and have attributed the onset of AD to insufficient sequestering of Abeta by TTR. We propose that TTR might be involved in the findings of RF exposure benefit in AD mice.

(E) Sokolovic D, Djindjic B, Nikolic J, Bjelakovic G, Pavlovic D, Kocic G, Krstic D, Cvetkovic T, Pavlovic V. Melatonin reduces oxidative stress induced by chronic exposure of microwave radiation from mobile phones in rat brain. J Radiat Res. 49(6):579-586, 2008. (AS, CE, CH, OX)

PURPOSE: The aim of the study was to evaluate the intensity of oxidative stress in the brain of animals chronically exposed to mobile phones and potential protective effects of melatonin in reducing oxidative stress and brain injury. **MATERIALS AND METHODS:** Experiments were performed on Wistar rats exposed to microwave radiation during 20, 40 and 60 days. Four groups were formed: I group (control)- animals treated by saline, intraperitoneally (i.p.) applied daily during follow up, II group (Mel)- rats treated daily with melatonin (2 mg kg(-1) body weight i.p.), III group (MWs)- microwave exposed rats, IV group (MWs + Mel)- MWs exposed rats treated with melatonin (2 mg kg(-1) body weight i.p.). The microwave radiation was produced by a mobile test phone (SAR = 0.043-0.135 W/kg). **RESULTS:** A significant increase in the brain tissue malondialdehyde (MDA) and carbonyl group concentration was registered during exposure. Decreased activity of catalase (CAT) and increased activity of xanthine oxidase (XO) remained after 40 and 60 days of exposure to mobile phones. Melatonin treatment significantly prevented the increase in the MDA content and XO activity in the brain tissue after 40 days of exposure while it was unable to prevent the decrease of CAT activity and increase of carbonyl group contents. **CONCLUSION:** We demonstrated two important findings; that mobile phones caused oxidative damage biochemically by increasing the levels of MDA, carbonyl groups, XO activity and decreasing CAT activity; and that treatment with the melatonin significantly prevented oxidative damage in the brain.

(E) Sokolovic D, Djordjevic B, Kocic G, Babovic P, Ristic G, Stanojkovic Z, Sokolovic DM, Veljkovic A, Jankovic A, Radovanovic Z. The effect of melatonin on body mass and behaviour of rats during an exposure to microwave radiation from mobile phone. Bratisl Lek Listy. 113(5):265-269, 2012. (AS, CE, PE, BE)

BACKGROUND: Microwave radiation (MW) produced by wireless telecommunications and a number of electrical devices used in household or in healthcare institutions may cause various disorders in human organism. On the other hand, melatonin is a potent antioxidant, immunostimulator and neuromodulator. The aim of this research was to determine body mass and behaviour changes in rats after a chronic microwave exposure, as well as to determine the effects of melatonin on body mass and behaviour in irradiated rats. METHODS: Wistar rats were divided into the four experimental groups: I group (control) - rats treated with 0,9 % saline, II group (Mel) - rats treated with melatonin (2 mg/kg), III group (MW) - rats exposed to MW radiation (4 h/day), IV group (MW+Mel) - rats, which were both exposed to MW radiation and received melatonin premedication (2 mg/kg). RESULTS: A significant body mass reduction was noted in animals exposed to MW radiation when compared to controls after 20, 40 and 60 days ($p<0.001$). Furthermore, body weight was significantly increased ($p<0.05$) in irradiated rats, which received melatonin pretreatment (MW+Mel) in comparison to irradiated group (MW) after 20 days. Microwave radiation exposed animals showed an anxiety related behaviour (agitation, irritability) after 10 days of exposure. After the radiation source removal, changes in behaviour were less noticeable. Melatonin administration to irradiated rats caused a decrease in the stress induced behaviour. CONCLUSION: Microwave radiation causes body mass decrease and anxiety related behaviour in rats, however melatonin causes a reverse of those effects on both body weight and behaviour of irradiated animals (Fig. 2, Ref. 32).

(E) Sonmez OF, Odaci E, Bas O, Kaplan S. Purkinje cell number decreases in the adult female rat cerebellum following exposure to 900 MHz electromagnetic field. Brain Res. 1356:95-101, 2010. (AS, CE, ME)

The biological effects of electromagnetic field (EMF) exposure from mobile phones have growing concern among scientists since there are some reports showing increased risk for human health, especially in the use of mobile phones for a long duration. In the presented study, the effects on the number of Purkinje cells in the cerebellum of 16-week (16 weeks) old female rats were investigated following exposure to 900 MHz EMF. Three groups of rats, a control group (CG), sham exposed group (SG) and an electromagnetic field exposed group (EMFG) were used in this study. While EMFG group rats were exposed to 900 MHz EMF (1h/day for 28 days) in an exposure tube, SG was placed in the exposure tube but not exposed to EMF (1h/day for 28 days). The specific energy absorption rate (SAR) varied between 0.016 (whole body) and 2 W/kg (locally in the head). The CG was not placed into the exposure tube nor was it exposed to EMF during the study period. At the end of the experiment, all of the female rats were sacrificed and the number of Purkinje cells was estimated using a stereological counting technique. Histopathological evaluations were also done on sections of the cerebellum. Results showed that the total number of Purkinje cells in the cerebellum of the EMFG was significantly lower than those of CG ($p<0.004$) and SG ($p<0.002$). In addition, there was no significant difference at the 0.05 level between the rats' body and brain weights in the EMFG and CG or SG. Therefore, it is suggested that long duration exposure to 900 MHz EMF leads to decreases of Purkinje cell numbers in the female rat cerebellum.

(E) Spichtig S, Scholkmann F, Chin L, Lehmann H, Wolf M. Assessment of intermittent UMTS electromagnetic field effects on blood circulation in the human auditory region using a near-infrared system. Bioelectromagnetics. 33(1):40-54, 2012. (HU, PE)

The aim of the present study was to assess the potential effects of intermittent Universal Mobile Telecommunications System electromagnetic fields (UMTS-EMF) on blood circulation in the human head (auditory region) using near-infrared spectroscopy (NIRS) on two different timescales: short-term (effects occurring within 80 s) and medium-term (effects occurring within 80 s to 30 min). For the first time, we measured potential immediate effects of UMTS-EMF in real-time without any interference during exposure. Three different exposures (sham, 0.18 W/kg, and 1.8 W/kg) were applied in a controlled, randomized, crossover, and double-blind paradigm on 16 healthy volunteers. In addition to oxy-, deoxy-, and total haemoglobin concentrations ([O(2) Hb], [HHb], and [tHb], respectively), the heart rate (HR), subjective well-being, tiredness, and counting speed were recorded. During exposure to 0.18 W/kg, we found a significant short-term increase in Δ[O(2) Hb] and Δ[tHb], which is small (≈17%) compared to a functional brain activation. A significant decrease in the medium-term response of Δ[HHb] at 0.18 and 1.8 W/kg exposures was detected, which is in the range of physiological fluctuations. The medium-term ΔHR was significantly higher (+1.84 bpm) at 1.8 W/kg than for sham exposure. The other parameters showed no significant effects. Our results suggest that intermittent exposure to UMTS-EMF has small short- and medium-term effects on cerebral blood circulation and HR.

(NE) Stefanics G, Kellényi L, Molnár F, Kubinyi G, Thuróczy G, Hernádi I. Short GSM mobile phone exposure does not alter human auditory brainstem response. BMC Public Health. 7:325, 2007. (HU, EE)

BACKGROUND: There are about 1.6 billion GSM cellular phones in use throughout the world today. Numerous papers have reported various biological effects in humans exposed to electromagnetic fields emitted by mobile phones. The aim of the present study was to advance our understanding of potential adverse effects of the GSM mobile phones on the human hearing system. **METHODS:** Auditory Brainstem Response (ABR) was recorded with three non-polarizing Ag-AgCl scalp electrodes in thirty young and healthy volunteers (age 18-26 years) with normal hearing. ABR data were collected before, and immediately after a 10 minute exposure to 900 MHz pulsed electromagnetic field (EMF) emitted by a commercial Nokia 6310 mobile phone. Fifteen subjects were exposed to genuine EMF and fifteen to sham EMF in a double blind and counterbalanced order. Possible effects of irradiation was analyzed by comparing the latency of ABR waves I, III and V before and after genuine/sham EMF exposure. **RESULTS:** Paired sample t-test was conducted for statistical analysis. Results revealed no significant differences in the latency of ABR waves I, III and V before and after 10 minutes of genuine/sham EMF exposure. **CONCLUSION:** The present results suggest that, in our experimental conditions, a single 10 minute exposure of 900 MHz EMF emitted by a commercial mobile phone does not produce measurable immediate effects in the latency of auditory brainstem waves I, III and V.

(NE) Stefanics G, Thuróczy G, Kellényi L, Hernádi I. Effects of twenty-minute 3G mobile phone irradiation on event related potential components and early gamma synchronization in auditory oddball paradigm. Neuroscience. 157(2):453-462, 2008. (HU, EE)

We investigated the potential effects of 20 min irradiation from a new generation Universal Mobile Telecommunication System (UMTS) 3G mobile phone on human event related potentials (ERPs) in an auditory oddball paradigm. In a double-blind task design, subjects were exposed to either genuine or sham irradiation in two separate sessions. Before and after irradiation subjects were presented with a random series of 50 ms tone burst (frequent standards: 1 kHz, P=0.8, rare deviants: 1.5 kHz, P=0.2) at a mean repetition rate of 1500 ms while electroencephalogram (EEG) was recorded. The subjects' task was to silently count the appearance of targets. The amplitude and latency of the N100, N200, P200 and P300 components for targets and standards were analyzed in 29 subjects. We found no significant effects of electromagnetic field (EMF) irradiation on the amplitude and latency of the above ERP components. In order to study possible effects of EMF on attentional processes, we applied a wavelet-based time-frequency method to analyze the early gamma component of brain responses to auditory stimuli. We found that the early evoked gamma activity was insensitive to UMTS RF exposition. Our results support the notion, that a single 20 min irradiation from new generation 3G mobile phones does not induce measurable changes in latency or amplitude of ERP components or in oscillatory gamma-band activity in an auditory oddball paradigm.

(NE) Stovner LJ, Oftedal G, Straume A, Johnsson A. Nocebo as headache trigger: evidence from a sham-controlled provocation study with RF fields. Acta Neurol Scand Suppl. 188:67-71, 2008. (HU, PE)

BACKGROUND: A large proportion of the population in Norway has experienced headache in connection with mobile phone use, but several double-blind provocation studies with radiofrequency (RF) and sham exposures have shown no relation between headache and mobile phone RF fields. AIMS: To investigate the type and location of headache experienced by participants in one provocation study in order to gain insight into possible causes and mechanisms of the headaches. METHOD: Questionnaire about headache, indication on figure of location of headache after exposure, interview with neurologist about headache features to make headache diagnoses. RESULTS: The 17 participants went through 130 trials (sham or RF exposure). No significant difference existed in headache type, laterality or location between the headaches experienced with the two exposures types. In most participants, the headache was compatible with tension-type headache. DISCUSSION: As participants experienced their typical 'mobile phone headache' both with and without RF exposure, and since the experiment did not involve the stress or the arm/head position of mobile phone use, the most likely explanation is that the headache in this situation is caused by negative expectations (nocebo). CONCLUSION: This and other similar studies indicate that headache occurring in connection with mobile phone use is not related to RF fields, and that a nocebo effect is important for this and possibly other headache triggers.

(E) **Sudan M, Kheifets L, Arah OA, Olsen J. Cell phone exposures and hearing loss in children in the Danish National Birth Cohort. Paediatr Perinat Epidemiol. 27(3):247-257, 2013. (HU, BE)**

BACKGROUND: Children today are exposed to cell phones early in life, and may be the most vulnerable if exposure is harmful to health. We investigated the association between cell phone use and hearing loss in children. METHODS: The Danish National Birth Cohort (DNBC) enrolled pregnant women between 1996 and 2002. Detailed interviews were conducted during gestation, and when the children were 6 months, 18 months and 7 years of age. We used multivariable-adjusted logistic regression, marginal structural models (MSM) with inverse-probability weighting, and doubly robust estimation (DRE) to relate hearing loss at age 18 months to cell phone use at age 7 years, and to investigate cell phone use reported at age 7 in relation to hearing loss at age 7. RESULTS: Our analyses included data from 52 680 children. We observed weak associations between cell phone use and hearing loss at age 7, with odds ratios and 95% confidence intervals from the traditional logistic regression, MSM and DRE models being 1.21 [95% confidence interval [CI] 0.99, 1.46], 1.23 [95% CI 1.01, 1.49] and 1.22 [95% CI 1.00, 1.49], respectively. CONCLUSIONS: Our findings could have been affected by various biases and are not sufficient to conclude that cell phone exposures have an effect on hearing. This is the first large-scale epidemiologic study to investigate this potentially important association among children, and replication of these findings is needed.

(NE) Terao Y, Okano T, Furubayashi T, Yugeta A, Inomata-Terada S, Ugawa Y. Effects of thirty-minute mobile phone exposure on saccades. Clin Neurophysiol. 118(7):1545-1556, 2007. (HU PE)

OBJECTIVE: To investigate whether exposure to pulsed high-frequency electromagnetic field (pulsed EMF) emitted by a mobile phone has short-term effects on saccade performances. METHODS: A double blind, counterbalanced crossover design was employed. In 10 normal subjects, we studied the performance of visually guided saccade (VGS), gap saccade (GAP), and memory guided saccade (MGS) tasks before and after exposure to EMF emitted by a mobile phone for thirty minutes or sham exposure. We also implemented a hand reaction time (RT) task in response to a visual signal. RESULTS: With the exception of VGS and MGS latencies, the parameters of VGS, GAP and MGS tasks were unchanged before and after real or sham EMF exposure. In addition, the latencies of VGS and MGS did not change differently after real and sham exposure. The hand RT shortened with the repetition of trials, but again this trend was of similar magnitude for real and sham exposures. CONCLUSIONS: Thirty minutes of mobile phone exposure has no significant short-term effect on saccade performances. SIGNIFICANCE: This is the first study to investigate saccade performance in relation to mobile phone exposure. No significant effect of mobile phone use was demonstrated on the performance of various saccade tasks, suggesting that the cortical processing for saccades and attention is not affected by exposure to EMF emitted by a mobile phone.

(NE) Thomas S, Benke G, Dimitriadis C, Inyang I, Sim MR, Wolfe R, Croft RJ, Abramson MJ. Use of mobile phones and changes in cognitive function in adolescents. Occup Environ Med. 67(12):861-866, 2010a. (HU, BE)

BACKGROUND: Several studies have investigated the impact of mobile phone exposure on cognitive function in adults. However, children and adolescents are of special interest due to their developing nervous systems. METHODS: Data were derived from the Australian Mobile Radiofrequency Phone Exposed Users' Study (MoRPhEUS) which comprised a baseline examination of year 7 students during 2005/2006 and a 1-year follow-up. Sociodemographic and exposure data were collected with a questionnaire. Cognitive functions were assessed with a computerised test battery and the Stroop Color-Word test. RESULTS: 236 students participated in both examinations. The proportion of mobile phone owners and the number of voice calls and short message services (SMS) per week increased from baseline to follow-up. Participants with more voice calls and SMS at baseline showed less reductions in response times over the 1-year period in various computerised tasks. Furthermore, those with increased voice calls and SMS exposure over the 1-year period showed changes in response time in a simple reaction and a working memory task. No associations were seen between mobile phone exposure and the Stroop test. CONCLUSIONS: We have observed that some changes in cognitive function, particularly in response time rather than accuracy, occurred with a latency period of 1 year and that some changes were associated with increased exposure. However, the increased exposure was mainly applied to those who had fewer voice calls and SMS at baseline, suggesting that these changes over time may relate to statistical regression to the mean, and not be the effect of mobile phone exposure.

(E) Thomas S, Heinrich S, von Kries R, Radon K. Exposure to radio-frequency electromagnetic fields and behavioural problems in Bavarian children and adolescents. Eur J Epidemiol. 25(2):135-141, 2010b. (HU, BE)

Only few studies have so far investigated possible health effects of radio-frequency electromagnetic fields (RF EMF) in children and adolescents, although experts discuss a potential higher vulnerability to such fields. We aimed to investigate a possible association between measured exposure to RF EMF fields and behavioural problems in children and adolescents. 1,498 children and 1,524 adolescents were randomly selected from the population registries of four Bavarian (South of Germany) cities. During an Interview data on participants' mental health, socio-demographic characteristics and potential confounders were collected. Mental health behaviour was assessed using the German version of the Strengths and Difficulties

Questionnaire (SDQ). Using a personal dosimeter, we obtained radio-frequency EMF exposure profiles over 24 h. Exposure levels over waking hours were expressed as mean percentage of the reference level. Overall, exposure to radiofrequency electromagnetic fields was far below the reference level. Seven percent of the children and 5% of the adolescents showed an abnormal mental behaviour. In the multiple logistic regression analyses measured exposure to RF fields in the highest quartile was associated to overall behavioural problems for adolescents (OR 2.2; 95% CI 1.1-4.5) but not for children (1.3; 0.7-2.6). These results are mainly driven by one subscale, as the results showed an association between exposure and conduct problems for adolescents (3.7; 1.6-8.4) and children (2.9; 1.4-5.9). As this is one of the first studies that investigated an association between exposure to mobile telecommunication networks and mental health behaviour more studies using personal dosimetry are warranted to confirm these findings.

(E) Thomée S, Härenstam A, Hagberg M. Mobile phone use and stress, sleep disturbances, and symptoms of depression among young adults--a prospective cohort study. BMC Public Health. 11:66, 2011. (HU, BE) (Effects may not be caused by RFR exposure.)

BACKGROUND: Because of the quick development and widespread use of mobile phones, and their vast effect on communication and interactions, it is important to study possible negative health effects of mobile phone exposure. The overall aim of this study was to investigate whether there are associations between psychosocial aspects of mobile phone use and mental health symptoms in a prospective cohort of young adults. METHODS: The study group consisted of young adults 20-24 years old (n = 4156), who responded to a questionnaire at baseline and 1-year follow-up. Mobile phone exposure variables included frequency of use, but also more qualitative variables: demands on availability, perceived stressfulness of accessibility, being awakened at night by the mobile phone, and personal overuse of the mobile phone. Mental health outcomes included current stress, sleep disorders, and symptoms of depression. Prevalence ratios (PRs) were calculated for cross-sectional and prospective associations between exposure variables and mental health outcomes for men and women separately. RESULTS: There were cross-sectional associations between high compared to low mobile phone use and stress, sleep disturbances, and symptoms of depression for the men and women. When excluding respondents reporting mental health symptoms at baseline, high mobile phone use was associated with sleep disturbances and symptoms of depression for the men and symptoms of depression for the women at 1-year follow-up. All qualitative variables had cross-sectional associations with mental health outcomes. In prospective analysis, overuse was associated with stress and sleep disturbances for women, and high accessibility stress was associated with stress, sleep disturbances, and symptoms of depression for both men and women. CONCLUSIONS: High frequency of mobile phone use at baseline was a risk factor for mental health outcomes at 1-year follow-up among the young adults. The risk for reporting mental health symptoms at follow-up was greatest among those who had perceived accessibility via mobile phones to be stressful. Public health prevention strategies focusing on attitudes could include information and advice, helping young adults to set limits for their own and others' accessibility.

(E) Tombini M, Pellegrino G, Pasqualetti P, Assenza G, Benvenga A, Fabrizio E, Rossini PM Mobile phone emissions modulate brain excitability in patients with focal epilepsy. Brain Stimul. 2012 Aug 9. [Epub ahead of print] (HU, EE, MA)

BACKGROUND: Electromagnetic fields (EMFs) emitted by mobile phones had been shown to increase cortical excitability in healthy subjects following 45 min of continuous exposure on the ipsilateral hemisphere. OBJECTIVE: Using Transcranial Magnetic Stimulation (TMS), the current study assessed the effects of acute exposure to mobile phone EMFs on the cortical excitability in patients with focal epilepsy. METHODS: Ten patients with cryptogenic focal epilepsy originating outside the primary motor area (M1) were studied. Paired-pulse TMS were applied to the M1 of both the hemisphere ipsilateral (IH) and contralateral (CH) to the epileptic focus before and immediately after real/sham exposure to the GSM-EMFs (45 min). The TMS study was carried out in all subjects in three different experimental sessions (IH and CH exposure, sham), 1 week apart, according to a crossover, double-blind and counter-balanced paradigm. RESULTS: The present study clearly demonstrated that an acute and relatively prolonged exposure to GSM-EMFs modulates cortical excitability in patients affected by focal epilepsy; however, in contrast to healthy subjects, these effects were evident only after EMFs exposure over the hemisphere contralateral to the epileptic focus (CH). They were characterized by a significant cortical excitability increase in the exposed hemisphere paired with slight excitability decrease in the other one (IH). Both sham and real EMFs exposure of the IH did not affect brain excitability. CONCLUSION: Present results suggest a significant interaction between the brain excitability changes induced by EMFs and the epileptic focus, which eliminated the excitability enhancing effects of EMFs evident only in the CH.

(E) Tong J, Chen S, Liu XM, Hao DM. [Effect of electromagnetic radiation on discharge activity of neurons in the hippocampus CA1 in rats]. Zhongguo Ying Yong Sheng Li Xue Za Zhi. 29(5):423-427, 2013. [Article in Chinese] (AS, CE, EE)

OBJECTIVE: In order to explore effect of electromagnetic radiation on learning and memory ability of hippocampus neuron in rats, the changes in discharge patterns and overall electrical activity of hippocampus neuron after electromagnetic radiation were observed. METHODS: Rat neurons discharge was recorded with glass electrode extracellular recording technology and a polygraph respectively. Radiation frequency of electromagnetic wave was 900 MHZ and the power was 10 W/m2. In glass electrode extracellular recording, the rats were separately irradiated for 10, 20, 30, 40, 50 and 60 min, every points repeated 10 times and updated interval of 1h, observing the changes in neuron discharge and spontaneous discharge patterns after electromagnetic radiation. In polygraph recording experiments, irradiation group rats for five days a week, 6 hours per day, repeatedly for 10 weeks, memory electrical changes in control group and irradiation group rats when they were feeding were repeatedly monitored by the implanted electrodes, observing the changes in peak electric digits and the largest amplitude in hippocampal CA1 area, and taking some electromagnetic radiation sampling sequence for correlation analysis. RESULTS: (1) Electromagnetic radiation had an inhibitory role on discharge frequency of the hippocampus CA1 region neurons. After electromagnetic radiation, discharge frequency of the hippocampus CA1 region neurons was reduced, but the changes in scale was not obvious. (2) Electromagnetic radiation might change the spontaneous discharge patterns of hippocampus CA1 region neurons, which made the explosive discharge pattern increased obviously. (3) Peak potential total number within 5 min in irradiation group was significantly reduced, the largest amplitude was less than that of control group. (4) Using mathematical method to make the correlation analysis of the electromagnetic radiation sampling sequence, that of irradiation group was less than that of control group, indicating that there was a tending to be inhibitory connection between neurons in irradiation group after electromagnetic radiation. CONCLUSION: Electromagnetic radiation may cause structure and function changes of transfer synaptic in global, make hippocampal CA1 area neurons change in the overall discharge characteristic and discharge patterns, thus lead to decrease in the ability of learning and memory.

(E) Trosić I, Pavicić I, Milković-Kraus S, Mladinić M, Zeljezić D. Effect of electromagnetic radiofrequency radiation on the rats' brain, liver and kidney cells measured by comet assay. Coll Antropol. 35(4):1259-1264, 2011. (AS, CE, CH)

The goal of study was to evaluate DNA damage in rat's renal, liver and brain cells after in vivo exposure to radiofrequency/microwave (Rf/Mw) radiation of cellular phone frequencies range. To determine DNA damage, a single cell gel electrophoresis/comet assay was used. Wistar rats (male, 12 week old, approximate body weight 350 g) (N = 9) were exposed to the carrier frequency of 915 MHz with Global System Mobile signal modulation (GSM), power density of 2.4 W/m2, whole body average specific absorption rate SAR of 0.6 W/kg. The animals were irradiated for one hour/day, seven days/week during two weeks period. The exposure set-up was Gigahertz Transversal Electromagnetic Mode Cell (GTEM--cell). Sham irradiated controls (N = 9) were apart of the study. The body temperature was measured before and after exposure. There were no differences in temperature in between control and treated animals. Comet assay parameters such as the tail length and tail intensity were evaluated. In comparison with tail length in controls (13.5 +/- 0.7 microm), the tail was slightly elongated in brain cells of irradiated animals (14.0 +/- 0.3 microm). The tail length obtained for liver (14.5 +/- 0.3 microm) and kidney (13.9 +/- 0.5 microm) homogenates notably differs in comparison with matched sham controls (13.6 +/- 0.3 microm) and (12.9 +/- 0.9 microm). Differences in tail intensity between control and exposed animals were not significant. The results of this study suggest that, under the experimental conditions applied, repeated 915 MHz irradiation could be a cause of DNA breaks in renal and liver cells, but not affect the cell genome at the higher extent compared to the basal damage.

(NE) Trunk A, Stefanics G, Zentai N, Kovács-Bálint Z, Thuróczy G, Hernádi I. No effects of a single 3G UMTS mobile phone exposure on spontaneous EEG activity, ERP correlates, and automatic deviance detection. Bioelectromagnetics. 2012 Jun 4. doi: 10.1002/bem.21740. [Epub ahead of print] (HU, EE)

Potential effects of a 30 min exposure to third generation (3G) Universal Mobile Telecommunications System (UMTS) mobile phone-like electromagnetic fields (EMFs) were investigated on human brain electrical activity in two experiments. In the first experiment, spontaneous electroencephalography (sEEG) was analyzed (n = 17); in the second experiment, auditory event-related potentials (ERPs) and automatic deviance detection processes reflected by mismatch negativity (MMN) were investigated in a passive oddball paradigm (n = 26). Both sEEG and ERP experiments followed a double-blind protocol where subjects were exposed to either genuine or sham irradiation in two separate sessions. In both experiments, electroencephalograms (EEG) were recorded at midline electrode sites before and after exposure while subjects were watching a silent documentary. Spectral power of sEEG data was analyzed in the delta, theta, alpha, and beta frequency bands. In the ERP experiment, subjects were presented with a random series of standard (90%) and frequency-deviant (10%) tones in a passive binaural oddball paradigm. The amplitude and latency of the P50, N100, P200, MMN, and P3a components were analyzed. We found no measurable effects of a 30 min 3G mobile phone irradiation on the EEG spectral power in any frequency band studied. Also, we found no significant effects of EMF irradiation on the amplitude and latency of any of the ERP components. In summary, the present results do not support the notion that a 30 min unilateral 3G EMF exposure interferes with human sEEG activity, auditory evoked potentials or automatic deviance detection indexed by MMN.

(NE) Unterlechner M, Sauter C, Schmid G, Zeitlhofer J. No effect of an UMTS mobile phone-like electromagnetic field of 1.97 GHz on human attention and reaction time. Bioelectromagnetics. 29(2):145-153, 2008. (HU, BE)

Several studies in the past reported influences of electromagnetic emissions of GSM phones on reaction time in humans. However, there are currently only a few studies available dealing with possible effects of the electromagnetic fields emitted by UMTS mobile phones. In our study, 40 healthy volunteers (20 female, 20 male), aged 26.0 years (range 21-30 years) underwent four different computer tests measuring reaction time and attention under three different UMTS mobile phone-like exposure conditions (two exposure levels plus sham exposure). Exposure of the subjects was accomplished by small helical antennas operated close to the head and fed by a generic signal representing the emissions of a UMTS mobile phone under constant receiving conditions as well as under a condition of strongly varying transmit power. In the high exposure condition the resulting peak spatial average exposure of the test subjects in the cortex of the left temporal lobe of the brain was 0.63 W/kg (min. 0.25 W/kg, max. 1.49 W/kg) in terms of 1 g averaged SAR and 0.37 W/kg (min. 0.16 W/kg, max. 0.84 W/kg) in terms of 10 g averaged SAR, respectively. Low exposure condition was one-tenth of high exposure and sham was at least 50 dB below low exposure. Statistical analysis of the obtained test parameters showed that exposure to the generic UMTS signal had no statistically significant immediate effect on attention or reaction. Therefore, this study does not provide any evidence that exposure of UMTS mobiles interferes with attention under short-term exposure conditions.

(E) Vácha M, Puzová T, Kvícalová M. Radio frequency magnetic fields disrupt magnetoreception in American cockroach. J Exp Biol. 212(Pt 21):3473-3477, 2009. (AS, LI, BE)

The sense that allows birds to orient themselves by the Earth's magnetic field can be disabled by an oscillating magnetic field whose intensity is just a fraction of the geomagnetic field intensity and whose oscillations fall into the medium or high frequency radio wave bands. This remarkable phenomenon points very clearly at one of two existing alternative magnetoreception mechanisms in terrestrial animals, i.e. the mechanism based on the radical pair reactions of specific photosensitive molecules. As the first such study in invertebrates, our work offers evidence that geomagnetic field reception in American cockroach is sensitive to a weak radio frequency field. Furthermore, we show that the 'deafening' effect at Larmor frequency 1.2 MHz is stronger than at different frequencies. The parameter studied was the rise in locomotor activity of cockroaches induced by periodic changes in the geomagnetic North positions by 60 deg. The onset of the disruptive effect of a 1.2 MHz field was found between 12 nT and 18 nT whereas the threshold of a doubled frequency field 2.4 MHz fell between 18 nT and 44 nT. A 7 MHz field showed no impact even in maximal 44 nT magnetic flux density. The results indicate resonance effects rather than non-specific bias of procedure itself and suggest that insects may be equipped with the same magnetoreception system as the birds.

(E) Vecchio F, Babiloni C, Ferreri F, Curcio G, Fini R, Del Percio C, Rossini PM. Mobile phone emission modulates interhemispheric functional coupling of EEG alpha rhythms. Eur J Neurosci. 25(6):1908-1913, 2007. (HU, EE)

We tested the working hypothesis that electromagnetic fields from mobile phones (EMFs) affect interhemispheric synchronization of cerebral rhythms, an important physiological feature of information transfer into the brain. Ten subjects underwent two electroencephalographic (EEG) recordings, separated by 1 week, following a crossover double-blind paradigm in which they were exposed to a mobile phone signal (global system for mobile communications; GSM). The mobile phone was held on the left side of the subject head by a modified helmet, and orientated in the normal position for use over the ear. The microphone was orientated towards the corner of the mouth, and the antenna was near the head in the parietotemporal area. In addition, we positioned another similar phone (but without battery) on the right side of the helmet, to balance the weight and to prevent the subject localizing the side of GSM stimulation (and consequently lateralizing attention). In one session the exposure was real (GSM) while in the other it was Sham; both sessions lasted 45 min. Functional interhemispheric connectivity was modelled using the analysis of EEG spectral coherence between frontal, central and parietal electrode pairs. Individual EEG rhythms of interest were delta (about 2-4 Hz), theta (about 4-6 Hz), alpha 1 (about 6-8 Hz), alpha 2 (about 8-10 Hz) and alpha 3 (about 10-12 Hz). Results showed that, compared to Sham stimulation, GSM stimulation modulated the interhemispheric frontal and temporal coherence at alpha 2 and alpha 3 bands. The present results suggest that prolonged mobile phone emission affects not only the cortical activity but also the spread of neural synchronization conveyed by interhemispherical functional coupling of EEG rhythms.

(E) Vecchio F, Buffo P, Sergio S, Iacoviello D, Rossini PM, Babiloni C. Mobile phone emission modulates event-related desynchronization of α rhythms and cognitive-motor performance in healthy humans. Clin Neurophysiol. 123(1):121-128, 2012a. (HU, EE, BE)

OBJECTIVES: It has been shown that electromagnetic fields of Global System for Mobile Communications phone (GSM-EMFs) affect human brain rhythms (Vecchio et al., 2007, 2010), but it is not yet clear whether these effects are related to alterations of cognitive functions. **METHODS:** Eleven healthy adults underwent two electroencephalographic (EEG) sessions separated by 1 week, following a cross-over, placebo-controlled, double-blind paradigm. In both sessions, they performed a visual go/no-go task before real exposure to GSM-EMFs or after a sham condition with no EMF exposure. In the GSM real session, temporal cortex was continuously exposed to GSM-EMFs for 45 min. In the sham session, the subjects were not aware that the EMFs had been switched off for the duration of the experiment. In the go/no-go task, a central fixation stimulus was followed by a green (50% of probability) or red visual stimulus. Subjects had to press the mouse button after the green stimuli (go trials). With reference to a baseline period, power decrease of low- (about 8-10 Hz) and high-frequency (about 10-12 Hz) alpha rhythms indexed the cortical activity. **RESULTS:** It was found less power decrease of widely distributed high-frequency alpha rhythms and faster reaction time to go stimuli in the post- than pre-exposure period of the GSM session. No effect was found in the sham session. **CONCLUSIONS:** These results suggest that the peak amplitude of alpha ERD and the reaction time to the go stimuli are modulated by the effect of the GSM-EMFs on the cortical activity. **SIGNIFICANCE:** Exposure to GSM-EMFs for 45 min may enhance human cortical neural efficiency and simple cognitive-motor processes in healthy adults.

(E) Vecchio F, Tombini M, Buffo P, Assenza G, Pellegrino G, Benvenga A, Babiloni C, Rossini PM. Mobile phone emission increases inter-hemispheric functional coupling of electroencephalographic alpha rhythms in epileptic patients. Int J Psychophysiol. 84(2):164-171, 2012b. (HU, EE, MA)

It has been reported that GSM electromagnetic fields (GSM-EMFs) of mobile phones modulate - after a prolonged exposure - inter-hemispheric synchronization of temporal and frontal resting electroencephalographic (EEG) rhythms in normal young and elderly subjects (Vecchio et al., 2007, 2010). Here we tested the hypothesis that this can be even more evident in epileptic patients, who typically suffer from abnormal mechanisms governing synchronization of rhythmic firing of cortical neurons. Eyes-closed resting EEG data were recorded in ten patients affected by focal epilepsy in real and sham exposure conditions. These data were compared with those obtained from 15 age-matched normal subjects of the previous reference studies. The GSM device was turned on (45 min) in the "GSM" condition and was turned off (45 min) in the other condition ("sham"). The mobile phone was always positioned on the left side in both patients and control subjects. Spectral coherence evaluated the inter-hemispheric synchronization of EEG rhythms at the following frequency bands: delta (about 2-4 Hz), theta (about 4-6 Hz), alpha1 (about 6-8 Hz), alpha2 (about 8-10 Hz), and alpha3 (about 10-12 Hz). The effects on the patients were investigated comparing the inter-hemispheric EEG coherence in the epileptic patients with the control group of subjects evaluated in the previous reference studies. Compared with the control subjects, epileptic patients showed a statistically significant higher inter-hemispheric coherence of temporal and frontal alpha rhythms (about 8-12 Hz) in the GSM than "Sham" condition. These results suggest that GSM-EMFs of mobile phone may affect inter-hemispheric synchronization of the dominant (alpha) EEG rhythms in epileptic patients. If confirmed by future studies on a larger group of epilepsy patients, the modulation of the inter-hemispheric alpha coherence due to the GSM-EMFs could have clinical implications and be related to changes in cognitive-motor function.

(E) Vecchio F, Babiloni C, Ferreri F, Buffo P, Cibelli G, Curcio G, van Dijkman S, Melgari JM, Giambattistelli F, Rossini PM. Mobile phone emission modulates inter-hemispheric functional coupling of EEG alpha rhythms in elderly compared to young subjects. Clin Neurophysiol. 121(2):163-171, 2010. (HU, EE, AD)

OBJECTIVE: It has been reported that GSM electromagnetic fields (GSM-EMFs) of mobile phones modulate--after a prolonged exposure--inter-hemispheric synchronization of temporal and frontal resting electroencephalographic (EEG) rhythms in normal young subjects [Vecchio et al., 2007]. Here we tested the hypothesis that this effect can vary on physiological aging as a sign of changes in the functional organization of cortical neural synchronization. **METHODS:** Eyes-closed resting EEG data were recorded in 16 healthy elderly subjects and 5 young subjects in the two conditions of the previous reference study. The GSM device was turned on (45 min) in one condition and was turned off (45 min) in the other condition. Spectral coherence evaluated the inter-hemispheric synchronization of EEG rhythms at the following bands: delta (about 2-4

Hz), theta (about 4-6 Hz), alpha 1 (about 6-8 Hz), alpha 2 (about 8-10 Hz), and alpha 3 (about 10-12 Hz). The aging effects were investigated comparing the inter-hemispheric EEG coherence in the elderly subjects vs. a young group formed by 15 young subjects (10 young subjects of the reference study; Vecchio et al., 2007). **RESULTS:** Compared with the young subjects, the elderly subjects showed a statistically significant ($p<0.001$) increment of the inter-hemispheric coherence of frontal and temporal alpha rhythms (about 8-12 Hz) during the GSM condition. **CONCLUSIONS:** These results suggest that GSM-EMFs of a mobile phone affect inter-hemispheric synchronization of the dominant (alpha) EEG rhythms as a function of the physiological aging. **SIGNIFICANCE:** This study provides further evidence that physiological aging is related to changes in the functional organization of cortical neural synchronization.

(E) Vecsei Z, Csathó A, Thuróczy G, Hernádi I. Effect of a single 30 min UMTS mobile phone**-like exposure on the thermal pain threshold of young healthy volunteers. Bioelectromagnetics. 2013 Jun 20. doi: 10.1002/bem.21801. [Epub ahead of print] (HU, BE)**

One of the most frequently investigated effects of radiofrequency electromagnetic fields (RF EMFs) on the behavior of complex biological systems is pain sensitivity. Despite the growing body of evidence of EMF-induced changes in pain sensation, there is no currently accepted experimental protocol for such provocation studies for the healthy human population. In the present study, therefore, we tested the effects of third generation Universal Mobile Telecommunications System (UMTS) RF EMF exposure on the thermal pain threshold (TPT) measured on the surface of the fingers of 20 young adult volunteers. The protocol was initially validated with a topical capsaicin treatment. The exposure time was 30 min and the genuine (or sham) signal was applied to the head through a patch antenna, where RF EMF specific absorption rate (SAR) values were controlled and kept constant at a level of 1.75 W/kg. Data were obtained using randomized, placebo-controlled trials in a double-blind manner. Subjective pain ratings were tested blockwise on a visual analogue rating scale (VAS). Compared to the control and sham conditions, the results provide evidence for intact TPT but a reduced desensitization effect between repeated stimulations within the individual blocks of trials, observable only on the contralateral side for the genuine UMTS exposure. Subjective pain perception (VAS) data indicated marginally decreased overall pain ratings in the genuine exposure condition only. The present results provide pioneering information about human pain sensation in relation to RF EMF exposure and thus may contribute to cover the existing gap between safety research and applied biomedical science targeting the potential biological effects of environmental RF EMFs.

(E) Velayutham P, Govindasamy GK, Raman R, Prepageran N, Ng KH. High-frequency hearing loss among mobile phone users. Indian J Otolaryngol Head Neck Surg. 2014 Jan;66(Suppl 1):169-72. doi: 10.1007/s12070-011-0406-4. Epub 2011 Dec 15. (HU, BE)

The objective of this study is to assess high frequency hearing (above 8 kHz) loss among prolonged mobile phone users is a tertiary Referral Center. Prospective single blinded study. This is the first study that used high-frequency audiometry. The wide usage of mobile phone is so profound that we were unable to find enough non-users as a control group. Therefore we compared the non-dominant ear to the dominant ear using audiometric measurements. The study was a blinded study wherein the audiologist did not know which was the dominant ear. A total of 100 subjects were studied. Of the subjects studied 53% were males and 47% females. Mean age was 27. The left ear was dominant in 63%, 22% were dominant in the right ear and 15% did not have a preference. This study showed that there is significant loss in the dominant ear compared to the non-dominant ear ($P < 0.05$). Chronic usage mobile phone revealed high frequency hearing loss in the dominant ear (mobile phone used) compared to the non dominant ear.

(E) Volkow ND, Tomasi D, Wang GJ, Vaska P, Fowler JS, Telang F, Alexoff D, Logan J, Wong C. Effects of cell phone radiofrequency signal exposure on brain glucose metabolism. JAMA. 305(8):808-813, 2011. (HU, PE)

CONTEXT: The dramatic increase in use of cellular telephones has generated concern about possible negative effects of radiofrequency signals delivered to the brain. However, whether acute cell phone exposure affects the human brain is unclear. **OBJECTIVE:** To evaluate if acute cell phone exposure affects brain glucose metabolism, a marker of brain activity. **DESIGN, SETTING, AND PARTICIPANTS:** Randomized crossover study conducted between January 1 and December 31, 2009, at a single US laboratory among 47 healthy participants recruited from the community. Cell phones were placed on the left and right ears and positron emission tomography with ((18)F)fluorodeoxyglucose injection was used to measure brain glucose metabolism twice, once with the right cell phone activated (sound muted) for 50 minutes ("on" condition) and once with both cell phones deactivated ("off" condition). Statistical parametric mapping was used to compare metabolism between on and off conditions using paired t tests, and Pearson linear correlations were used to verify the association of metabolism and estimated amplitude of radiofrequency-modulated electromagnetic waves emitted by the cell phone. Clusters with at least 1000 voxels (volume >8 cm(3)) and $P < .05$ (corrected for multiple comparisons) were considered significant. **MAIN OUTCOME MEASURE:** Brain glucose metabolism computed as absolute metabolism (µmol/100 g per minute) and as normalized metabolism (region/whole brain). **RESULTS:** Whole-brain metabolism did not differ between on and off conditions. In contrast, metabolism in the region closest to the antenna (orbitofrontal cortex and temporal pole) was significantly higher for on than off conditions (35.7 vs 33.3 µmol/100 g per minute; mean difference, 2.4 [95% confidence interval, 0.67-4.2]; $P = .004$). The increases were significantly correlated with the estimated electromagnetic field amplitudes both for absolute metabolism ($R = 0.95$, $P < .001$) and normalized metabolism ($R = 0.89$; $P < .001$). **CONCLUSIONS:** In healthy participants and compared with no exposure, 50-minute cell phone exposure was associated with increased brain glucose metabolism in the region closest to the antenna. This finding is of unknown clinical significance.

(NE) Wallace D, Eltiti S, Ridgewell A, Garner K, Russo R, Sepulveda F, Walker S, Quinlan T, Dudley S, Maung S, Deeble R, Fox E. Cognitive and physiological responses in humans exposed to a TETRA base station signal in relation to perceived electromagnetic hypersensitivity. Bioelectromagnetics. 33(1):23-39, 2012. (HU, BE)

Terrestrial Trunked Radio (TETRA) technology ("Airwave") has led to public concern because of its potential interference with electrical activity in the brain. The present study is the first to examine whether acute exposure to a TETRA base station signal has an impact on cognitive functioning and physiological responses. Participants were exposed to a 420 MHz TETRA signal at a power flux density of 10 mW/m(2) as well as sham (no signal) under double-blind conditions. Fifty-one people who reported a perceived sensitivity to electromagnetic fields as well as 132 controls participated in a double-blind provocation study. Forty-eight sensitive and

132 control participants completed all three sessions. Measures of short-term memory, working memory, and attention were administered while physiological responses (blood volume pulse, heart rate, skin conductance) were monitored. After applying exclusion criteria based on task performance for each aforementioned cognitive measure, data were analyzed for 36, 43, and 48 sensitive participants for these respective tasks and, likewise, 107,125, and 129 controls. We observed no differences in cognitive performance between sham and TETRA exposure in either group; physiological response also did not differ between the exposure conditions. These findings are similar to previous double-blind studies with other mobile phone signals (900-2100 MHz), which could not establish any clear evidence that mobile phone signals affect health or cognitive function.

(E) Wang H, Peng R, Zhou H, Wang S, Gao Y, Wang L, Yong Z, Zuo H, Zhao L, Dong J, Xu X, Su Z. Impairment of long-term potentiation induction is essential for the disruption of spatial memory after microwave exposure. Int J Radiat Biol. 2013 Jul 24. [Epub ahead of print] (AS, BE, ME, EE)

Purpose: To assess the impact of microwave exposure on learning and memory and to explore the underlying mechanisms. Materials and methods: 100 Wistar rats were exposed to a 2.856 GHz pulsed microwave field at average power densities of 0 mW/cm^2, 5 mW/cm^2, 10 mW/cm^2 and 50 mW/cm^2 for 6 min. The spatial memory was assessed by the Morris Water Maze (MWM) task. An in vivo study was conducted soon after microwave exposure to evaluate the changes of population spike (PS) amplitudes of long-term potentiation (LTP) in the medial perforant path (MPP)-dentate gyrus (DG) pathway. The structure of the hippocampus was observed by the light microscopy and the transmission electron microscopy (TEM) at 7 d after microwave exposure. Results: Our results showed that the rats exposed in 10 mW/cm^2 and 50 mW/cm^2 microwave displayed significant deficits in spatial learning and memory at 6 h, 1 d and 3 d after exposure. Decreased PS amplitudes were also found after 10 mW/cm^2 and 50 mW/cm^2 microwave exposure. In addition, varying degrees of degeneration of hippocampal neurons, decreased synaptic vesicles and blurred synaptic clefts were observed in the rats exposed in 10 mW/cm^2 and 50 mW/cm^2 microwave. Compared with the sham group, the rats exposed in 5 mW/cm^2 microwave showed no difference in the above experiments. Conclusions: This study suggested that impairment of LTP induction and the damages of hippocampal structure, especially changes of synapses, might contribute to cognitive impairment after microwave exposure.

(NE) Watilliaux A, Edeline JM, Lévêque P, Jay TM, Mallat M. Effect of exposure to 1,800 MHz electromagnetic fields on heat shock proteins and glial cells in the brain of developing rats. Neurotox Res. 20(2):109-119, 2011. (AS, DE, CC)

The increasing use of mobile phones by children raise issues about the effects of electromagnetic fields (EMF) on the immature Central Nervous System (CNS). In the present study, we quantified cell stress and glial responses in the brain of developing rats one day after a single exposure of 2 h to a GSM 1,800 MHz signal at a brain average Specific Absorption Rate (SAR) in the range of 1.7 to 2.5 W/kg. Young rats, exposed to EMF on postnatal days (P) 5 (n = 6), 15 (n = 5) or 35 (n = 6), were compared to pseudo-exposed littermate rats (n = 6 at all ages). We used western blotting to detect heat shock proteins (HSPs) and cytoskeleton- or neurotransmission-related proteins in the developing astroglia. The GSM signal had no significant effect on the abundance of HSP60, HSC70 or HSP90, of serine racemase, glutamate transporters including GLT1 and GLAST, or of glial fibrillary acid protein (GFAP) in either total or soluble tissue extracts. Imunohistochemical detection of CD68 antigen in brain sections from pseudo-exposed and exposed animals did not reveal any differences in the morphology or distribution of microglial cells. These results provide no evidence for acute cell stress or glial reactions indicative of early neural cell damage, in developing brains exposed to 1,800 MHz signals in the range of SAR used in our study.

(E) Wiholm C, Lowden A, Kuster N, Hillert L, Arnetz BB, Akerstedt T, Moffat SD.Mobile phone exposure and spatial memory. Bioelectromagnetics. 30(1):59-65, 2009. (HU, BE)

Radiofrequency (RF) emission during mobile phone use has been suggested to impair cognitive functions, that is, working memory. This study investigated the effects of a 2 1/2 h RF exposure (884 MHz) on spatial memory and learning, using a double-blind repeated measures design. The exposure was designed to mimic that experienced during a real-life mobile phone conversation. The design maximized the exposure to the left hemisphere. The average exposure was peak spatial specific absorption rate (psSAR10g) of 1.4 W/kg. The primary outcome measure was a "virtual" spatial navigation task modeled after the commonly used and validated Morris Water Maze. The distance traveled on each trial and the amount of improvement across trials (i.e., learning) were used as dependent variables. The participants were daily mobile phone users, with and without symptoms attributed to regular mobile phone use. Results revealed a main effect of RF exposure and a significant RF exposure by group effect on distance traveled during the trials. The symptomatic group improved their performance during RF exposure while there was no such effect in the non-symptomatic group. Until this new finding is further investigated, we can only speculate about the cause.

(E) Xu S, Zhou Z, Zhang L, Yu Z, Zhang W, Wang Y, Wang X, Li M, Chen Y, Chen C, He M, Zhang G, Zhong M. Exposure to 1800 MHz radiofrequency radiation induces oxidative damage to mitochondrial DNA in primary cultured neurons. Brain Res. 1311:189-196, 2010. (CS, CH, OX)

Increasing evidence indicates that oxidative stress may be involved in the adverse effects of radiofrequency (RF) radiation on the brain. Because mitochondrial DNA (mtDNA) defects are closely associated with various nervous system diseases and mtDNA is particularly susceptible to oxidative stress, the purpose of this study was to determine whether radiofrequency radiation can cause oxidative damage to mtDNA. In this study, we exposed primary cultured cortical neurons to pulsed RF electromagnetic fields at a frequency of 1800 MHz modulated by 217 Hz at an average special absorption rate (SAR) of 2 W/kg. At 24 h after exposure, we found that RF radiation induced a significant increase in the levels of 8-hydroxyguanine (8-OHdG), a common biomarker of DNA oxidative damage, in the mitochondria of neurons. Concomitant with this finding, the copy number of mtDNA and the levels of mitochondrial RNA (mtRNA) transcripts showed an obvious reduction after RF exposure. Each of these mtDNA disturbances could be reversed by pretreatment with melatonin, which is known to be an efficient antioxidant in the brain. Together, these results suggested that 1800 MHz RF radiation could cause oxidative damage to mtDNA in primary cultured neurons. Oxidative damage to mtDNA may account for the neurotoxicity of RF radiation in the brain.

(E) Yan JG, Agresti M, Zhang LL, Yan Y, Matloub HS. Upregulation of specific mRNA levels in rat brain after cell phone exposure. Electromagn Biol Med. 27(2):147-154, 2008. (AS, CE, CH)

Adult Sprague-Dawley rats were exposed to regular cell phones for 6 h per day for 126 days (18 weeks). RT-PCR was used to investigate the changes in levels of mRNA synthesis of several injury-associated proteins. Calcium ATPase, Neural Cell Adhesion Molecule, Neural Growth Factor, and Vascular Endothelial Growth Factor were evaluated. The results showed statistically significant mRNA up-regulation of these proteins in the brains of rats exposed to cell phone radiation. These results indicate that relative chronic exposure to cell phone microwave radiation may result in cumulative injuries that could eventually lead to clinically significant neurological damage.

(E) Yang XS, He GL, Hao YT, Xiao Y, Chen CH, Zhang GB, Yu ZP. Exposure to 2.45 GHz electromagnetic fields elicits an HSP-related stress response in rat hippocampus. Brain Res Bull. 88(4):371-378, 2012. (AS, CH)

The issue of possible neurobiological effects of the electromagnetic field (EMF) exposure is highly controversial. To determine whether electromagnetic field exposure could act as an environmental stimulus capable of producing stress responses, we employed the hippocampus, a sensitive target of electromagnetic radiation, to assess the changes in its stress-related gene and protein expression after EMF exposure. Adult male Sprague-Dawley rats with body restrained were exposed to a 2.45 GHz EMF at a specific absorption rate (SAR) of 6 W/kg or sham conditions. cDNA microarray was performed to examine the changes of gene expression involved in the biological effects of electromagnetic radiation. Of 2048 candidate genes, 23 upregulated and 18 downregulated genes were identified. Of these differential expression genes, two heat shock proteins (HSP), HSP27 and HSP70, are notable because expression levels of both proteins are increased in the rat hippocampus. Result from immunocytochemistry revealed that EMF caused intensive staining for HSP27 and HSP70 in the hippocampus, especially in the pyramidal neurons of cornu ammonis 3 (CA3) and granular cells of dentate gyrus (DG). The gene and protein expression profiles of HSP27 and HSP70 were further confirmed by reverse transcription polymerase chain reaction (RT-PCR) and Western blot. Our data provide direct evidence that exposure to electromagnetic fields elicits a stress response in the rat hippocampus.

(NE) Yilmaz F, Dasdag S, Akdag MZ, Kilinc N. Whole-body exposure of radiation emitted from 900 MHz mobile phones does not seem to affect the levels of anti-apoptotic bcl-2 protein. Electromagn Biol Med. 27(1):65-72, 2008. (AS, CH)

The purpose of the present study was to investigate the anti-apoptotic bcl-2 protein in rat brain and testes after whole-body exposure to radiation emitted from 900 MHz cellular phones. Two groups (sham and experimental) of Sprague-Dawley rats of eight rats each were used in the study. Exposure began approximately 10 min after transferring into the exposure cages, a period of time when rats settled down to a prone position and selected a fixed location inside the cage spontaneously. For the experimental group, the phones were in the speech condition for 20 min per day for 1 month. The same procedure was applied to the sham group rats, but the phones were turned off. Immunohistochemical staining of bcl-2 was performed according to the standardized avidin-biotin complex method. The results of this study showed that 20 min of the radiation emitted from 900 MHz cellular phones did not alter anti-apoptotic bcl-2 protein in the brain and testes of rats. We speculate that bcl-2 may not be involved in the effects of radiation on the brain and testes of rats.

***(E) Yuan K, Qin W, Wang G, Zeng F, Zhao L, Yang X, Liu P, Liu J, Sun J, von Deneen KM, Gong Q, Liu Y, Tian J. Microstructure abnormalities in adolescents with internet addiction disorder. PLoS One. 6(6):e20708, 2011. (HU, ME) (*Effects observed probably not caused by exposure to RFR.)**

BACKGROUND: Recent studies suggest that internet addiction disorder (IAD) is associated with structural abnormalities in brain gray matter. However, few studies have investigated theeffects of internet addiction on the microstructural integrity of major neuronal fiber pathways, and almost no studies have assessed the microstructural changes with the duration of internet addiction. **METHODOLOGY/PRINCIPAL FINDINGS:** We investigated the morphology of the brain in adolescents with IAD (N=18) using an optimized voxel-based morphometry (VBM) technique, and studied the white matter fractional anisotropy (FA) changes using the diffusion tensor imaging (DTI) method, linking these brain structural measures to the duration of IAD. We provided evidences demonstrating the multiple structural changes of the brain in IAD subjects. VBM results indicated the decreased gray matter volume in the bilateral dorsolateral prefrontal cortex (DLPFC), the supplementary motor area (SMA), the orbitofrontal cortex (OFC), the cerebellum and the left rostral ACC (rACC). DTI analysis revealed the enhanced FA value of the left posterior limb of the internal capsule (PLIC) and reduced FA value in the white matter within the right parahippocampal gyrus (PHG). Gray matter volumes of the DLPFC, rACC, SMA, and white matter FA changes of the PLIC were significantly correlated with the duration of internet addiction in the adolescents with IAD. **CONCLUSIONS:** Our results suggested that long-term internet addiction would result in brain structural alterations, which probably contributed to chronic dysfunction in subjects with IAD. The current study may shed further light on the potential brain effects of IAD.

(E) Zareen N, Khan MY, Ali Minhas L. Derangement of chick embryo retinal differentiation caused by radiofrequency electromagnetic fields. Congenit Anom (Kyoto). 49(1):15-19, 2009. (AS, CE, ME, DE)

The possible adverse effects of radiofrequency electromagnetic fields (EMF) emitted from mobile phones present a major public concern. Biological electrical activities of the human body are vulnerable to interference from oscillatory aspects of EMF, which affect fundamental cellular activities, in particular, the highly active development process of embryos. Some studies highlight the possible health hazards of EMF, while others contest the hypothesis of biological impact of EMF. The present study was designed to observe the histomorphological effects of EMF emitted by a mobile phone on the retinae of developing chicken embryos. Fertilized chicken eggs were exposed to a ringing mobile set on silent tone placed in the incubator at different ages of development. After exposure for the scheduled duration the retinae of the embryos were dissected out and processed for histological examination. The control and experimental embryos were statistically compared for retinal thickness and epithelial pigmentation grades. Contrasting effects of EMF on the retinal histomorphology were noticed, depending on the duration of exposure. The embryos exposed for 10 post-incubation days exhibited decreased retinal growth and mild pigmentation of the epithelium. Growth retardation reallocated to growth enhancement on increasing EMF exposure for 15 post-incubation days, with a shift of pigmentation grade from mild to intense. We conclude that EMF emitted by a mobile phone cause derangement of chicken embryo retinal differentiation.

(E) Zhang SZ, Yao GD, Lu DQ, Chiang H, Xu ZP. [Effect of 1.8 GHz radiofrequency electromagnetic fields on gene expression of rat neurons]. Zhonghua Lao Dong Wei Sheng Zhi Ye Bing Za Zhi. 26(8):449-452, 2008. [Article in Chinese] (CS, CH, WS)

OBJECTIVE: To investigate the changes of gene expression in rat neuron induced by 1.8 GHz radiofrequency electromagnetic fields (RF EMF) to screen for RF EMF-responsive genes and the effect of different exposure times and modes on the gene expression in neuron. METHODS: Total RNA was extracted immediately and purified from the primary culture of neurons after intermittent exposed or sham-exposed to a frequency of 1.8 GHz RF EMF for 24 hours at an average special absorption rate (SAR) of 2 W/kg. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron. Differentially expressed genes (Egr-1, Mbp and Plp) were further confirmed by semi-quantitative revere transcription polymerase chain reaction (RT PCR). The expression levels of Egr-1, Mbp and Plp were observed at different exposure times (6, 24 h) and modes (intermittent and continuous exposure). RESULTS: Among 1200 candidate genes, 24 up-regulated and 10 down-regulated genes were found by using Affymetrix microarray suite software 5.0 which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. Under 24 h and 6 h intermittent exposure, Egr-1 and Plp in experiment groups showed statistic significance ($P < 0.05$) compared with the control groups, while expression of Mbp did not change significantly ($P > 0.05$). After 24 h continuous exposure, Egr-1 and Mbp in experiment groups showed statistic significance ($P < 0.05$) compared with the control group, while expression of Plp did not change significantly ($P > 0.05$). Under the same exposure mode 6 h, expression of all the 3 genes did not change significantly. Different times (6, 24 h) and modes (intermittent and continuous exposure) of exposure exerted remarkable different influences on the expression of Egr-1, Mbp, Plp genes ($P < 0.01$). CONCLUSION: The changes of many genes transcription were involved in the effect of 1.8 GHz RF EMF on rat neurons; Down-regulation of Egr-1 and up-regulation of Mbp, Plp indicated the negative effects of RF EMF on neurons; The effect of RF intermittent exposure on gene expression was more obvious than that of continuous exposure; The effect of 24 h RF exposure (both intermittent and continuous) on gene expression was more obvious than that of 6 h (both intermittent and continuous).

(E) Zhang Y, She F, Li L, Chen C, Xu S, Luo X, Li M, He M, Yu Z. p25/CDK5 is partially involved in neuronal injury induced by radiofrequency electromagnetic field exposure. Int J Radiat Biol. 2013 Jul 29. [Epub ahead of print] (CS, CC)

Purpose: Several studies suggest that radiofrequency electromagnetic field (RF-EMF) exposure can induce neuronal injury. The aim of the present work was to investigate whether the cyclin-dependent kinase 5 (CDK5) pathway is involved in neuronal injury induced by RF-EMF exposure. Materials and methods: Newborn Sprague-Dawley rats' primary cultured cortical neurons were exposed to pulsed 2.45 GHz RF-EMF for 10 min. The cellular viability was assessed using the 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay. The apoptosis was assessed by Hoechst 33342 and terminal deoxynucleotidyl transferase (TdT)-mediated dUTP nick-end labeling co-staining. The protein expressions of CDK5, p35, p25, and phosphorylated tau at Ser^{404} were examined by Western blot analysis. The CDK5 activity was detected using a histone-H1 kinase assay. Results: The cellular viability of neurons was significantly decreased ($p < 0.01$, Partial Eta Squared [η_p^2]: 0.554), and the percentage of apoptotic nuclei ($p < 0.01$, $\eta_p^2 = 0.689$), activity of CDK5 ($p < 0.05$, $\eta_p^2 = 0.589$), ratio of p25 and p35 ($p < 0.05$, $\eta_p^{2\,=}$ 0.670), levels of tau phosphorylation at Ser^{404} ($p < 0.01$, $\eta_p^2 = 0.896$) were significantly increased after RF-EMF exposure. No significant change was detected in CDK5 expression after RF-EMF exposure. Pretreatment with Roscovitine (a CDK5 inhibitor) significantly blocked the RF-EMF-induced decrease of cellular viability ($p < 0.05$, $\eta_p^2 = 0.398$) and tau hyperphosphorylation at Ser^{404} ($p < 0.01$, $\eta_p^{2\,=}$ 0.917), but did not significantly block the RF-EMF-induced apoptosis ($p > 0.05$, $\eta_p^2 = 0.130$). Conclusions: These results suggest that abnormal activity of p25/CDK5 is partially involved in primary cultured cortical neuron injury induced by RF-EMF exposure.

(E) Zhao R, Zhang S, Xu Z, Ju L, Lu D, Yao G. Studying gene expression profile of rat neuron exposed to 1800MHz radiofrequency electromagnetic fields with cDNA microassay. Toxicology. 235(3):167-175, 2007. (CS, CH)

A widespread use of mobile phone (MP) evokes a growing concern for their possible adverse effects on human, especially the brain. Gene expression is a unique way of characterizing how cells and organism adapt to changes in the external environment, so the aim of this investigation was to determine whether 1800 MHz radiofrequency electromagnetic fields (RF EMF) can influence the gene expression of neuron. Affymetrix Rat Neurobiology U34 array was applied to investigate the changes of gene expression in rat neuron after exposed to the pulsed RF EMF at a frequency of 1800 MHz modulated by 217 Hz which is commonly used in MP. Among 1200 candidate genes, 24 up-regulated genes and 10 down-regulated genes were identified after 24-h intermittent exposure at an average special absorption rate (SAR) of 2 W/kg, which are associated with multiple cellular functions (cytoskeleton, signal transduction pathway, metabolism, etc.) after functional classification. The results were further confirmed by quantitative real-time polymerase chain reaction (RT PCR). The present results indicated that the gene expression of rat neuron could be altered by exposure to RF EMF under our experimental conditions.

(E) Zhao TY, Zou SP, Knapp PE. Exposure to cell phone radiation up-regulates apoptosis genes in primary cultures of neurons and astrocytes. Neurosci Lett. 412(1):34-38, 2007. (CS, CH)

The health effects of cell phone radiation exposure are a growing public concern. This study investigated whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to a working Global System for Mobile Communication (GSM) cell phone rated at a frequency of 1900MHz. Primary cultures were exposed to cell phone emissions for 2h. We used array analysis and real-time RT-PCR to show up-regulation of caspase-2, caspase-6 and Asc (apoptosis associated speck-like protein containing a card) gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons, but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The effects are specific since up-regulation was not seen for other genes associated with apoptosis, such as caspase-9 in either neurons or astrocytes, or Bax in neurons. The results show that even relatively short-term exposure to cell phone radiofrequency emissions can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

APPENDIX B: ABSTRACTS OF STUDIES ON NEUROLOGICAL EFFECTS OF EXTREMELY-LOW FREQUENCY (EMF) - (2007-2014)

Keys: (E) - effect observed; (NE) -no significant effect observed.
AS- animal study; CS- cell/in vitro study; CE- chronic/repeated exposure; AE- acute exposure; HU- human study; MC- morphological changes; CC- chemical changes; FC- functional changes; EE- electrophysiological changes; BE- changes in behavior; OX- oxidative changes; DE- development; MA- possible medical application; ND- neurodegenerative disease; EF- electric field.

(E) Ahmed Z, Wieraszko A. The mechanism of magnetic field-induced increase of excitability in hippocampal neurons. Brain Res. 1221:30-40, 2008. (CS, AE, EE)

The influence of a pulsed magnetic field (PMF) on hippocampal evoked potentials has been investigated in vitro. The exposure to PMF (0.16 Hz, 15 mT) applied for 30 min amplified the population spike and the slope of EPSP recorded from stratum pyramidale and stratum radiatum respectively. This amplification was additive to previously induced LTP and occurred in an NMDA-independent way. The increase in the activity of electrical synapses accompanied PMF-induced amplification of evoked potentials. Since PMF exposure modified paired-pulse facilitation and paired-pulse inhibition, it was concluded that it modifies excitatory and inhibitory processes in the hippocampus. Control experiments revealed that observed effects were exclusively related to PMF exposure. The results support and extend our previous research indicating a significant influence of magnetic fields on hippocampal physiology.

(E) Akdag MZ, Dasdag S, Ulukaya E, Uzunlar AK, Kurt MA, Taşkin A. Effects of extremely low-frequency magnetic field on caspase activities and oxidative stress values in rat brain. Biol Trace Elem Res. 138(1-3):238-249, 2010. (OX, AS, CC, CE)

This study was aimed to investigate the effect of extremely low-frequency magnetic field (ELF-MF) on apoptosis and oxidative stress values in the brain of rat. Rats were exposed to 100 and 500 μT ELF-MF, which are the safety standards of public and occupational exposure for 2 h/day for 10 months. Brain tissues were immunohistochemically stained for the active (cleaved) caspase-3 in order to measure the apoptotic index by a semi-quantitative scoring system. In addition, the levels of catalase (CAT), malondialdehyde (MDA), myeloperoxidase (MPO), total antioxidative capacity (TAC), total oxidant status (TOS), and oxidative stress index (OSI) were measured in rat brain. Final score of apoptosis and MPO activity were not significantly different between the groups. CAT activity decreased in both exposure groups ($p < 0.05$), while TAC was found to be lower in ELF 500 group than those in ELF-100 and sham groups ($p < 0.05$). MDA, TOS, and OSI values were found to be higher in ELF-500 group than those in ELF-100 and sham groups ($p < 0.05$). In conclusion, apoptosis was not changed by long-term ELF-MF exposure, while both 100 and 500 μT ELF-MF exposure induced toxic effect in the rat brain by increasing oxidative stress and diminishing antioxidant defense system.

(E) Akdag MZ, Dasdag S, Cakir DU, Yokus B, Kizil G, Kizil M. Do 100- and 500-μT ELF magnetic fields alter beta-amyloid protein, protein carbonyl and malondialdehyde in rat brains? Electromagn Biol Med. 32(3):363-372, 2013. (AS, CE, CC, OX)

Several studies still state that presently accepted safety standards for extremely low-frequency magnetic fields (ELF-MFs) do not provide adequate protection, and therefore the standards are still open to question. To help resolve this question, the aim of this study was to illuminate the interaction between biomolecules and ELF-MFs by investigating the effect of ELF-MFs on beta-amyloid protein (BAP), protein carbonyl (PC) and malondialdehyde (MDA) in rat brain. For this study, 30 adult male Sprague-Dawley rats were used, which were divided into two experimental groups and a sham exposed group. Rats in two experimental groups were exposed to 100- and 500-μT ELF-MFs (50 Hz) for 2 h/day for 10 months, which are the generally accepted safety standards for public and occupational exposures. The same procedures were applied to the rats in the sham group, but with the generator turned off. The results of this study showed that neither ELF-MFs used in this study altered BAP level significantly ($p>0.05$). However, PC and MDA levels were increased by the exposure to 100- and 500-μT ELF-MFs ($p < 0.0001$). In conclusion, both PC and MDA levels were altered by long-term exposure to either 100 or 500 μT ELF-MF. However, many further and more comprehensive studies will be required to elucidate the interaction mechanisms between ELF-MFs exposure and living organisms.

(E) Akpinar D, Ozturk N, Ozen S, Agar A, Yargicoglu P. The effect of different strengths of extremely low-frequency electric fields on antioxidant status, lipid peroxidation, and visual evoked potentials. Electromagn Biol Med. 31(4):436-448, 2012. (AS, CE, OX, EE)

The aim of the study was to investigate the effects of extremely low-frequency electric field (ELF EF) on visual evoked potential (VEP), thiobarbituric acid reactive substances (TBARS), total antioxidant status (TAS), total oxidant status (TOS), and oxidant stress index (OSI). Thirty female Wistar rats, aged 3 months, were divided into three equal groups: Control (C), the group exposed to EF at 12 kV/m strength (E12), and the group exposed to EF at 18 kV/m strength (E18). Electric field was applied to the E12 and E18 groups for 14 days (1 h/day). Brain and retina TBARS, TOS, and OSI were significantly increased in the E12 and E18 groups with respect to the control group. Also, TBARS levels were significantly increased in the E18 group compared with the E12 group. Electric fields significantly decreased TAS levels in both brain and retina in E12 and E18 groups with respect to the control group. All VEP components were significantly prolonged in rats exposed to electric fields compared to control group. In addition, all latencies of VEP components were increased in the E18 group with respect to the E12 group. It is conceivable to suggest that EF-induced lipid peroxidation may play an important role in changes of VEP parameters.

(NE) Aldinucci C, Carretta A, Maiorca SM, Leoncini S, Signorini C, Ciccoli L, Pessina GP. Effects of 50 Hz electromagnetic fields on rat cortical synaptosomes. Toxicol Ind Health. 25(4-5):249-252, 2009. (CS, CC, AE)

Nerve cells are very responsive to weak pulsed electromagnetic fields (EMFs). Such non-ionizing radiation, with frequencies of 0-300 Hz and 0.1-100 mT, can affect several cellular activities, with unusual dose-response characteristics. The present study examined the effect of a 2-h exposure of synaptosomes on a system generating a peak magnetic field of 2 mT. We evaluated the changes of the synaptosomal mitochondrial respiration rate and ATP production, membrane potential, intrasynaptosomal Ca^{2+} concentration, and the release of free iron and

F2-isoprostanes. O2 consumption and ATP production remained unchanged in exposed synaptosomes. The intrasynaptosomal Ca2+ concentration decreased slowly and no depolarization of the synaptosomal membrane was detected. Finally, the release of free iron and F2-isoprostanes by synaptosomal suspensions also remained unchanged after EMF exposure. These results indicate that the physiological behavior of cortical synaptosomes was unaffected by weak pulsed EMFs.

(E) Amirifalah Z, Firoozabadi SM, Shafiei SA. Local Exposure of Brain Central Areas to a Pulsed ELF Magnetic Field for a Purposeful Change in EEG. Clin EEG Neurosci. 44(1):44-52, 2013. (HU, EE)

This study examines the simultaneous exposure of 2 brain areas in the location of central electrodes (C3 and C4) to a weak and pulsed extremely low-frequency magnetic field (ELF-MF) on the electroencephalogram (EEG). The intent is to change the EEG for a therapeutic application, such as neurofeedback, by inducing the "resonance effect." A total of 10 healthy women received 9 minutes of ELF-MF (intensity 200 µT) and sham in a counterbalanced design. ELF-MF exposure frequencies were 10, 14, and 18 Hz. The paired t test revealed that local pulsed ELF-MF significantly decreases beta (15-25 Hz), sensorimotor rhythm (13-15 Hz), and theta (4-8 Hz) powers at a frequency of 10 Hz in C3 and C4 regions (12.0%-26.6%) after exposure, in comparison with that achieved during the exposure ($P < .05$). Variations during the exposure were transient and different from those after. The resonance effect was observed nowhere around the regions. The study suggests that this technique may be applied in the treatment of anxiety; however, further investigation is needed.

(NE) Azanza MJ, del Moral A, Calvo AC, Pérez-Bruzón RN, Junquera C. Synchronization dynamics induced on pairs of neurons under applied weak alternating magnetic fields. Comp Biochem Physiol A Mol Integr Physiol. 166(4):603-618, 2013.(CS, AE, EE, MC)

Pairs of Helix aspersa neurons show an alternating magnetic field dependent frequency synchronization (AMFS) when exposed to a weak (amplitude B0 between 0.2 and 150 Gauss (G)) alternating magnetic field (AMF) of extremely low frequency (ELF, fM = 50 Hz). We have compared the AMFS patterns of discharge with: i) the synaptic activity promoted by glutamate and acetylcholine; ii) the activity induced by caffeine; iii) the bioelectric activity induced on neurons interconnected by electric synapses. AMFS activity reveals several specific features: i) a tight coincidence in time of the pattern and frequency, f, of discharge; ii) it is induced in the time interval of field application; iii) it is dependent on the intensity of the sinusoidal applied magnetic field; iv) elicited biphasic responses (excitation followed by inhibition) run in parallel for the pair of neurons; and v) some neuron pairs either spontaneously or AMF synchronized can be desynchronized under applied higher AMF. Our electron microscopy studies reveal gap-like junctions confirming our immunocytochemistry results about expression of connexin 26 (Cx26) in 4.7% of Helix neurons. AMF and carbenoxolone did not induce any significant effect on spontaneous synchronization through electric synapses.

(E) Bai WF, Xu WC, Feng Y, Huang H, Li XP, Deng CY, Zhang MS. Fifty-Hertz electromagnetic fields facilitate the induction of rat bone mesenchymal stromal cells to

differentiate into functional neurons. Cytotherapy. 15(8):961-970, 2013. (CS, CE, MC, MA, ND)

BACKGROUND AIMS: Research results have shown that bone mesenchymal stromal cells (BMSC) can different into neural cells. Electromagnetic fields (EMF) play a role in regulating cell proliferation and differentiation, but the mechanisms behind this are unknown. In the present study, we explored the efficacy of EMF on the induction of rat BMSC differentiation into neurons in vitro. METHODS: First, rat BMSC were induced in a nerve cell culture environment and divided into three groups: an EMF induction treatment group (frequency of 50 Hz, magnetic induction of 5 mT, 60 min per day for 12 days), an induction-only group and a control group. Second, we observed cell phenotypes in a confocal microscope, tested gene expression through the use of reverse transcriptase-polymerase chain reaction, and detected postsynaptic currents by means of a cell patch-clamp. We analyzed the cell cycles and the portion of cells expressing neural cell markers with the use of flow cytometry. RESULTS: The results indicated that EMF can facilitate BMSC differentiation into neural cells, which expressed neuronal-specific markers and genes; they formed synaptic junctions and pulsed excitatory postsynaptic currents. At the same time, the G0-G1 phase ratio recorded by means of flow cytometry gradually decreased under the EMF treatment, whereas there was an increase of S-phase ratio, and the portion of cells expressing neuronal-specific markers increased. CONCLUSIONS: Given that a noninvasive treatment of 50-Hz EMF could significantly facilitate BMSC to differentiate into functional neurons, EMF appears to be a promising clinical option for stem cell transplantation therapies to combat central nervous system diseases.

(E) Balassa T, Szemerszky R, Bárdos G. Effect of short-term 50 Hz electromagnetic field exposure on the behavior of rats. Acta Physiol Hung. 96(4):437-448, 2009. (AS, BE, AE)

Extremely low-frequency electromagnetic field generated by transformer stations located within buildings has been suspected to initiate non-specific health problems. This possibility was examined in model experiments in rats. Following short-term exposure (50 Hz, 500 mircoT, 20 min), situational and social anxiety as well as locomotor activity pattern were examined by several different tests (elevated plus-maze, novel object exploration, social interaction and territoriality).Based on our results having obtained so far, it seems that these field parameters (that equals the official reference limit for workers) may cause some kind of discomfort, may influence behavior, increase passivity and situational anxiety, but has no verified effect on the social and territorial behavior.

(E) Balassa T, Varró P, Elek S, Drozdovszky O, Szemerszky R, Világi I, Bárdos G. Changes in synaptic efficacy in rat brain slices following extremely low-frequency magnetic field exposure at embryonic and early postnatal age. Int J Dev Neurosci. 31(8):724-730, 2013. (AS, CE, EE, DE)

An earlier study demonstrated changes in synaptic efficacy and seizure susceptibility in adult rat brain slices following extremely low-frequency magnetic field (ELF-MF) exposure. The developing embryonic and early postnatal brain may be even more sensitive to MF exposure. The aim of the present study was to determine the effects of a long-term ELF-MF (0.5 and 3 mT, 50 Hz) exposure on synaptic functions in the developing brain. Rats were treated with chronic exposure to MF during two critical periods of brain development, i.e. in utero during the second gestation week or as newborns for 7 days starting 3 days after birth, respectively. Excitability and plasticity of neocortical and hippocampal areas were tested on brain slices by analyzing extracellular evoked field potentials. We demonstrated that the basic excitability of hippocampal slices (measured as amplitude of population spikes) was increased by both types of treatment (fetal 0.5 mT, newborn 3 mT). Neocortical slices seemed to be responsive mostly to the newborn treatment, the amplitude of excitatory postsynaptic potentials was increased. Fetal ELF-MF exposure significantly inhibited the paired-pulse depression (PPD) and there was a significant decrease in the efficacy of LTP (long-term potentiation induction) in neocortex, but not in hippocampus. On the other hand, neonatal treatment had no significant effect on plasticity phenomena. Results demonstrated that ELF-MF has significant effects on basic neuronal functions and synaptic plasticity in brain slice preparations originating from rats exposed either in fetal or in newborn period.

(E) Calabrò E, Condello S, Magazù S, Ientile, R. Static and 50 Hz electromagnetic fields effects on human neuronal-like cells vibration bands in the mid-infrared region. J Electromagnetic Analysis and Applications 3(2) 69-78, 2011. (CS, AE, CC)

Human neuronal-like cells were exposed to static and 50 Hz electromagnetic fields at the intensities of 2 mT and 1 mT, respectively. The effects of exposure were investigated in the mid-infrared region by means of Fourier self deconvolution spectroscopic analysis. After exposure of 3 hours to static and 50 Hz electromagnetic fields, the vibration bands of CH2 methilene group increased significantly after both exposures, suggesting a relative increase of lipid related to conformational changes in the cell membrane due to electromagnetic fields. In addition, PO2- stretching phosphate bands decreased after both exposures, suggesting that alteration in DNA/RNA can be occurred. In particular, exposure of 3 hours to 50 Hz electromagnetic fields produced significant increases in β-sheet contents in amide I, and around the 1740 cm^{-1} band assigned to non-hydrogen-bonded ester carbonyl stretching mode, that can be related to unfolding processes of proteins structure and cells death. Further exposure up to 18 hours to static magnetic field produced an increase in β-sheet contents as to α-helix components of amide I region, as well.

(E) Calabrò E, Condello S, Currò M, Ferlazzo N, Vecchio M, Caccamo D, Magazù S, Ientile R. 50 Hz Electromagnetic Field Produced Changes in FTIR Spectroscopy Associated with Mitochondrial Transmembrane Potential Reduction in Neuronal-Like SH-SY5Y Cells. Oxid Med Cell Longev. 2013;2013:414393. doi: 10.1155/2013/414393. Epub 2013 Jul 16. (CS, AE, EE)

SH-SY5Y neuroblastoma cells were used as an experimental model to study the effects of 50 Hz electromagnetic field, in the range from 50 μ T to 1.4 mT. Fourier transform infrared spectroscopy analysis evidenced a reduction in intensity of the amide A band and a slight increase of vibration bands at 2921 cm(-1) and 2853 cm(-1) corresponding to methylene groups. A further increase of the magnetic field intensity of exposure up to 0.8 mT and 1.4 mT produced a clear increase in intensity of CH2 vibration bands. Moreover, it has been observed some alterations in the amide I region, such as a shifted peak of the amide I band to a smaller wavenumber, probably due to protein conformational changes. These results suggested that exposure to extremely low electromagnetic fields influenced lipid components of cellular membrane and the N-H in-plane bending and C-N stretching vibrations of peptide linkages, modifying the secondary structures of α -helix and β -sheet contents and producing unfolding process in cell membrane proteins. The observed changes after exposure to 50 Hz electromagnetic field higher than 0.8 mT were associated with a significant reduction of cell viability and reduced mitochondrial transmembrane potential.

(NE) Canseven AG, Keskil ZA, Keskil S, Seyhan N. Pentylenetetrazol-induced seizures are not altered by pre- or post-drug exposure to a 50 Hz magnetic field. Radiat Biol. 83(4):231-235, 2007. (AS, AE, BE)

PURPOSE: To investigate whether pre- and post-drug magnetic field (MF) exposure of 50 Hz, 0.2 mT has any significant effect on pentylenetetrazol (PTZ)-induced seizures in mice. MATERIAL AND METHODS: MF was generated by a pair of Helmholtz coils. Seizures were induced by PTZ injection intraperitoneally (i.p.) at a dose of 60 mg/kg. A total of 48 locally bred adult female mice 25-35 g in weight were used. Latency to seizure, total seizure duration, and mortality were recorded for each mouse. RESULTS: Neither pre- nor post-drug exposure to a 50 Hz, 0.2 mT MF was found to have any effect on PTZ-induced epileptic seizures or mortality rates in mice. CONCLUSION: The present study failed to provide any support for a therapeutic potential of a 50 Hz, 0.2 mT MF for epilepsy.

(E) Capone F, Dileone M, Profice P, Pilato F, Musumeci G, Minicuci G, Ranieri F, Cadossi R, Setti S, Tonali PA, Di Lazzaro V. Does exposure to extremely low frequency magnetic fields produce functional changes in human brain? J Neural Transm. 116(3):257-265, 2009. (HU, FC)

Behavioral and neurophysiological changes have been reported after exposure to extremely low frequency magnetic fields (ELF-MF) both in animals and in humans. The physiological bases of these effects are still poorly understood. In vitro studies analyzed the effect of ELF-MF applied in pulsed mode (PEMFs) on neuronal cultures showing an increase in excitatory neurotransmission. Using transcranial brain stimulation, we studied noninvasively the effect of PEMFs on several measures of cortical excitability in 22 healthy volunteers, in 14 of the subjects we also evaluated the effects of sham field exposure. After 45 min of PEMF exposure, intracortical facilitation produced by paired pulse brain stimulation was significantly enhanced with an increase of about 20%, while other parameters of cortical excitability remained unchanged. Sham field exposure produced no effects. The increase in paired-pulse facilitation, a physiological parameter related to cortical glutamatergic activity, suggests that PEMFs exposure may produce an enhancement in cortical excitatory neurotransmission. This study suggests that PEMFs may produce functional changes in human brain.

(E) Carrubba S, Frilot C 2nd, Chesson AL Jr, Marino AA. Mobile-phone pulse triggers evoked potentials. Neurosci Lett. 469(1):164-168, 2010. (HU, EE)

If mobile-phone electromagnetic fields (EMFs) are hazardous, as suggested in the literature, processes or mechanisms must exist that allow the body to detect the fields. We hypothesized that the low-frequency pulses produced by mobile phones (217 Hz) were detected by sensory transduction, as evidenced by the ability of the pulses to trigger evoked potentials (EPs). Electroencephalograms (EEGs) were recorded from six standard locations in 20 volunteers and analyzed to detect brain potentials triggered by a pulse of the type produced by mobile phones. Evoked potentials having the expected latency were found in 90% of the volunteers, as assessed using a nonlinear method of EEG analysis. Evoked potentials were not detected when the EEG was analyzed using time averaging. The possibility of systematic error was excluded by sham-exposure analyses. The results implied that mobile-phones trigger EP at the rate of 217 Hz during ordinary phone use. Chronic production of the changes in brain activity might be pertinent to the reports of health hazards among mobile-phone users.

(E) Carrubba S, Frilot C, Chesson AL, Marino AA. Nonlinear EEG activation evoked by low-strength low-frequency magnetic fields. Neurosci Lett. 417(2):212-216, 2007. (HU, AE, EE)

Recent electrophysiological evidence suggested the existence of a human magnetic sense, but the kind of dynamical law that governed the stimulus-response relationship was not established. We tested the hypothesis that brain potentials evoked by the onset of a weak, low-frequency magnetic field were nonlinearly related to the stimulus. A field of 1G, 60 Hz was applied for 2s, with a 5s inter-stimulus period, and brain potentials were recorded from occipital electrodes in eight subjects, each of whom were measured twice, with at least 1 week between measurements. The recorded signals were subjected to nonlinear (recurrence analysis) and linear (time averaging) analyses. Using recurrence analysis, magnetosensory evoked potentials (MEPs) were detected in each subject in both the initial and replicate studies, with one exception. All MEPs exhibited the expected latency but differed in dynamical characteristics, indicating that they were nonlinearly related to the stimulus. MEPs were not detected using time averaging, thereby further confirming their nonlinearity. Evolutionarily conditioned structures that help mediate linear field-transduction in lower life forms may be expressed and functionally utilized in humans, but in a role where they facilitate vulnerability to man-made environmental fields.

(E) Celik MS, Guven K, Akpolat V, Akdag MZ, Naziroglu M, Gul-Guven R, Celik MY, Erdogan S. Extremely low-frequency magnetic field induces manganese accumulation in brain, kidney and liver of rats. Toxicol Ind Health. 2013 Feb 28. [Epub ahead of print] (AS, CE, CC)

The aim of the present study was to determine the effects of extremely low-frequency magnetic field (ELF-MF) on accumulation of manganese (Mn) in the kidney, liver and brain of rats. A total of 40 rats were randomly divided into eight groups. Four control groups received 0, 3.75, 15 and 60 mg Mn per kg body weight orally every 2 days for 45 days, respectively. The remaining four groups received same concentrations of Mn and were also exposed to ELF-MF (1.5 mT; 50 Hz) for 4 h for 5 days a week during 45 days. Following the last exposure, kidney, liver and brain were taken from all rats and they were analyzed for Mn accumulation levels using an inductively coupled plasma-optical emission spectrometer. In result of the current study, we observed that Mn levels in brain, kidney and liver were higher in Mn groups than in control groups. Mn levels in brain, kidney and liver were also higher in Mn plus ELF-MF groups than in Mn groups. In conclusion, result of the current study showed that the ELF-MF induced manganese accumulation in kidney, liver and brain of rats.

(E) Che Y, Sun H, Cui Y, Zhou D, Ma Y. Effects of exposure to 50 Hz magnetic field of 1 mT on the performance of detour learning task by chicks. Brain Res Bull. 74(1-3):178-182, 2007. (AS, CE, BE)

In the present study, we examined the effects of exposure to an extremely low-frequency magnetic field of 1 mT intensity on learning and memory in Lohmann brown domestic chicks using detour learning task. These results show that 20 h/day exposure to a low-frequency magnetic field induces a significant impairment in detour learning but 50 min/day exposure has no effect.

(E) Cho H, Seo YK, Yoon HH, Kim SC, Kim SM, Song KY, Park JK. Neural stimulation on human bone marrow-derived mesenchymal stem cells by extremely low frequency electromagnetic fields (ELF-EMFs). Biotechnol Prog. 2012 Jul 31. doi: 10.1002/btpr.1607. [Epub ahead of print] (CS, CE, MC, DE, MA)

Adult stem cells are considered to be multipotent.Especially, human bone marrow-derived mesenchymal stem cells (hBM-MSCs) have the potential to differentiate into nerve type cells. Electromagnetic fields (EMFs) are widely distributed in the environment, and recently there have been many reports on the biological effects of EMFs. hBM-MSCs are weak and sensitive pluripotent stem cells, therefore extremely low frequency- electromagnetic fields (ELF-EMFs) could be affect the changes of biological functions within the cells. In our experiments, ELF-EMFs inhibited the growth of hBM-MSCs in 12 days exposure. Their gene level was changed and expression of the neural stem cell marker like nestin was decreased but the neural cell markers like MAP2, NEUROD1, NF-L and Tau were induced. In immunofluorescence study, we confirmed the expression of each protein of neural cells. And also both oligodendrocyte and astrocyte related proteinslike O4 and GFAP were expressed by ELF-EMFs. **We suggest that EMFs can induce neural differentiation in BM-MSCs without any chemicals or differentiation factors.**

(E) Cho SI, Nam YS, Chu LY, Lee JH, Bang JS, Kim HR, Kim HC, Lee YJ, Kim HD, Sul JD, Kim D, Chung YH, Jeong JH. Extremely low-frequency magnetic fields modulate nitric oxide signaling in rat brain. Bioelectromagnetics. 33(7):568-574, 2012. (AS, CE, CC, OX)

Our previous study has shown that an extremely low-frequency magnetic field (ELF-MF) induces nitric oxide (NO) synthesis by Ca(2+) -dependent NO synthase (NOS) in rat brain. The present study was designed to confirm that ELF-MF affects neuronal NOS (nNOS) in several brain regions and to investigate the correlation between NO and nNOS activation. The exposure of rats to a 2 mT, 60 Hz ELF-MF for 5 days resulted in increases of NO levels in parallel with cGMP elevations in the cerebral cortex, striatum, and hippocampus. Cresyl violet staining and electron microscopic evaluation revealed that there were no significant differences in the morphology and number of neurons in the cerebral cortex, striatum, and hippocampus. Differently, the numbers of nNOS-immunoreactive (IR) neurons were significantly increased in those cerebral areas in ELF-MF-exposed rats. These data suggest that the increase in NO could be due to the increased expression and activation of nNOS in cells. Based on NO signaling in physiological and pathological states, ELF-MF created by electric power systems may induce various physiological changes in modern life.

(E) Chu LY, Lee JH, Nam YS, Lee YJ, Park WH, Lee BC, Kim D, Chung YH, Jeong JH. Extremely low frequency magnetic field induces oxidative stress in mouse cerebellum. Gen Physiol Biophys. 30(4):415-421, 2011. (AS, CE, OX)

We have investigated whether extremely low frequency magnetic field (ELF-MF) induces lipid peroxidation and reactive oxygen species in mouse cerebellum. After exposure to 60 Hz ELF-MF at 2.3 mT intensity for 3 hours, there was a significant increase in malondialdehyde level and hydroxyl radical. ELF-MF significantly induced concomitant increase in superoxide dismutase without alteration in glutathione peroxidase activity. While glutathione contents were not altered, ascorbic acid levels were significantly decreased by ELF-MF exposure. These results indicate that ELF-MF may induce oxidative stress in mouse cerebellum. However, the mechanism remains further to be characterized.

(E) Ciejka E, Kleniewska P, Skibska B, Goraca A. Effects of extremely low frequency magnetic field on oxidative balance in brain of rats. J Physiol Pharmacol. 62(6):657-661, 2011. (AS, CE, OX)

Extremely low frequency magnetic field (ELF-MF) may result in oxidative DNA damage and lipid peroxidation with an ultimate effect on a number of systemic disturbances and cell death. The aim of the study is to assess the effect of ELF-MF parameters most frequently used in magnetotherapy on reactive oxygen species generation (ROS) in brain tissue of experimental animals depending on the time of exposure to this field. The research material included adult male Sprague-Dawley rats, aged 3-4 months. The animals were divided into 3 groups: I - control (shame) group; II - exposed to the following parameters of the magnetic field: 7 mT, 40 Hz, 30 min/day, 10 days; III - exposed to the ELF-MF parameters of 7 mT, 40 Hz, 60 min/day, 10 days. The selected parameters of oxidative stress: thiobarbituric acid reactive substances (TBARS), hydrogen peroxide (H(2)O(2)), total free sulphydryl groups (-SH groups) and protein in brain homogenates were measured after the exposure of rats to the magnetic field. ELF-MF parameters of 7 mT, 40 Hz, 30 min/day for 10 days caused a significant increase in lipid peroxidation and insignificant increase in H(2)O(2) and free -SH groups. The same ELF-MF parameters but applied for 60 min/day caused a significant increase in free -SH groups and protein concentration in the brain homogenates indicating the adaptive mechanism. The study has shown that ELF-MF applied for 30 min/day for 10 days can affect free radical generation in the brain. Prolongation of the exposure to ELF-MF (60/min/day) caused adaptation to this field. The effect of ELF-MF irradiation on oxidative stress parameters depends on the time of animal exposure to magnetic field.

(E) Cook CM, Saucier DM, Thomas AW, Prato FS. Changes in human EEG alpha activity following exposure to two different pulsed magnetic field sequences. Bioelectromagnetics. 30(1):9-20, 2009. (AE, HU, EE)

The present study investigates the effects of a weak (+/-200 microT(pk)), pulsed, extremely low frequency magnetic field (ELF MF) upon the human electroencephalogram (EEG). We have previously determined that exposure to pulsed ELF MFs can affect the EEG, notably the alpha frequency (8-13 Hz) over the occipital-parietal region of the scalp. In the present study, subjects (n = 32) were exposed to two different pulsed MF sequences (1 and 2, used previously) that differed in presentation rate, in order to examine the effects upon the alpha frequency of the human EEG. Results suggest that compared to sham exposure, alpha activity was lowered over the occipital-parietal regions of the brain during exposure to Sequence 1, while alpha activity over the same regions was higher after Sequence 2 exposure. These effects occurred after approximately 5 min of pulsed MF exposure. The results also suggest that a previous exposure to the pulsed MF sequence determined subjects' responses in the present experiment. This study supports our previous observation of EEG changes after 5 min pulsed ELF MF exposure. The results of this study are also consistent with existing EEG experiments of ELF MF and mobile phone effects upon the brain.

(E) Corbacio M, Brown S, Dubois S, Goulet D, Prato FS, Thomas AW, Legros A. Human cognitive performance in a 3 mT power-line frequency magnetic field. Bioelectromagnetics. 32(8):620-633, 2011. (HU, AE, BE)

Extremely low frequency (ELF, <300 Hz) magnetic fields (MF) have been reported to modulate cognitive performance in humans. However, little research exists with MF exposures comparable to the highest levels experienced in occupations like power line workers and industrial welders. This research aims to evaluate the impact of a 60 Hz, 3 mT MF on human cognitive performance. Ninety-nine participants completed the double-blind protocol, performing a selection of psychometric tests under two consecutive MF exposure conditions dictated by assignment to one of three groups (sham/sham, MF exposure/sham, or sham/MF exposure). Data were analyzed using a 3 × 2 mixed model analysis of variance. Performance between repetitions improved in 11 of 15 psychometric parameters (practice effect). A significant interaction effect on the digit span forward test ($F = 5.21$, $P < 0.05$) revealed an absence of practice effects for both exposure groups but not the control group. **This memory test indicates MF-induced abolition of the improvement associated with practice**. Overall, this study does not establish any clear MF effect on human cognition. It is speculated that an ELF MF may interfere with the neuropsychological processes responsible for this short-term learning effect supported by brain synaptic plasticity.

(E) Coşkun S, Balabanli B, Canseven A, Seyhan N. Effects of continuous and intermittent magnetic fields on oxidative parameters in vivo. Neurochem Res. 34(2):238-243, 2009. (AS, CE, CC, OX)

Continuous and intermittent 50 Hz, 1.5 mT magnetic field with the exposure period of 4 h/day for 4 days was used to investigate its possible effect on adult guinea pigs. Tissues and plasma specimens were assessed by biochemical parameters. Malondialdehyde (MDA), glutathione (GSH), nitric oxide (NO) levels and myeloperoxidase activity (MPO) were examined in plasma, liver and brain tissues. All parameters were determined by spectrophotometer. While intermittent magnetic field was effective on plasma lipid peroxidation, continuous magnetic field was found to be effective on plasma MPO activity and NO levels. Augmentation of lipid peroxidation was also observed in liver tissue both intermittent and continuous magnetic field exposures. These results indicate that both the intermittent and continuous magnetic field exposures affect various tissues in a distinct manner because of having different tissue antioxidant status and responses.

(E) Cuccurazzu B, Leone L, Podda MV, Piacentini R, Riccardi E, Ripoli C, Azzena GB, Grassi C. Exposure to extremely low-frequency (50 Hz) electromagnetic fields enhances adult hippocampal neurogenesis in C57BL/6 mice. Exp Neurol. 226(1):173-182, 2010. (AS, CE, MC, MA)

Throughout life, new neurons are continuously generated in the hippocampus, which is therefore a major site of structural plasticity in the adult brain. We recently demonstrated that extremely low-frequency electromagnetic fields (ELFEFs) promote the neuronal differentiation of neural stem cells in vitro by up-regulating Ca(v)1-channel activity. The aim of the present study was to determine whether 50-Hz/1 mT ELFEF stimulation also affects adult hippocampal neurogenesis in vivo, and if so, to identify the molecular mechanisms underlying this action and its functional impact on synaptic plasticity. ELFEF exposure (1 to 7 h/day for 7 days) significantly enhanced neurogenesis in the dentate gyrus (DG) of adult mice, as documented by increased numbers of cells double-labeled for 5-bromo-deoxyuridine (BrdU) and double cortin. Quantitative RT-PCR analysis of hippocampal extracts revealed significant ELFEF exposure-induced increases in the transcription of pro-neuronal genes (Mash1, NeuroD2, Hes1) and genes encoding Ca(v)1.2 channel α(1C) subunits. Increased expression of NeuroD1, NeuroD2 and Ca(v)1 channels was also documented by Western blot analysis. Immunofluorescence experiments showed that, 30 days after ELFEF stimulation, roughly half of the newly generated immature neurons had survived and become mature dentate granule cells (as shown by their immunoreactivity for both BrdU and NeuN) and were integrated into the granule cell layer of the DG. Electrophysiological experiments demonstrated that the new mature neurons influenced hippocampal synaptic plasticity, as reflected by increased long-term potentiation. Our findings show that ELFEF exposure can be an effective tool for increasing in vivo neurogenesis, and they could lead to the development of novel therapeutic approaches in regenerative medicine.

(E) Cui Y, Ge Z, Rizak JD, Zhai C, Zhou Z, Gong S, Che Y. Deficits in water maze performance and oxidative stress in the hippocampus and striatum induced by extremely low frequency magnetic field exposure. PLoS One. 7(5):e32196, 2012. (AS, CE, BE, OX)

The exposures to extremely low frequency magnetic field (ELF-MF) in our environment have dramatically increased. Epidemiological studies suggest that there is a possible association between ELF-MF exposure and increased risks of cardiovascular disease, cancers and neurodegenerative disorders. Animal studies show that ELF-MF exposure may interfere with the activity of brain cells, generate behavioral and cognitive disturbances, and produce deficits in attention, perception and spatial learning. Although, many research efforts have been focused on the interaction between ELF-MF exposure and the central nervous system, the mechanism of interaction is still unknown. In this study, we examined the effects of ELF-MF exposure on learning in mice using two water maze tasks and on some parameters indicative of oxidative stress in the hippocampus and striatum. We found that ELF-MF exposure (1 mT, 50 Hz) induced serious oxidative stress in the hippocampus and striatum and impaired hippocampal-dependent spatial learning and striatum-dependent habit learning. This study provides evidence for the association between the impairment of learning and the oxidative stress in hippocampus and striatum induced by ELF-MF exposure.

(E) Cvetkovic D, Cosic I. Alterations of human electroencephalographic activity caused by multiple extremely low frequency magnetic field exposures. Med Biol Eng Comput. 47(10):1063-1073, 2009. (HU, AE, EE, MA)

In the past, many studies have claimed that extremely low frequency (ELF) magnetic field (MF) exposures could alter the human electroencephalographic (EEG) activity. This study aims at extending our ELF pilot study to investigate whether MF exposures at ELF in series from 50, 16.66, 13, 10, 8.33 to 4 Hz could alter relative power within the corresponding EEG bands. 33 human subjects were tested under a double-blind and counter-balanced conditions. The multiple repeated three-way analysis of variance (ANOVA) mixed design (within and between-subject) analysis was employed followed by post-hoc t-tests and Bonferroni alpha-correction. The results from this study have shown that narrow alpha1 (7.5-9.5 Hz) and alpha2 (9-11 Hz) bands, associated with 8.33 and 10 Hz MF exposures, were significantly ($p < 0.0005$) lower than control over the temporal and parietal regions within the 10-16 min of first MF exposure session and the MF exposures were significantly higher than control of the second session MF exposure (60-65 min from the commencement of testing). Also, it was found that the beta1 (12-14 Hz) band exhibited a significant increase from before to after 13-Hz first MF exposure session at frontal region. The final outcome of our result has shown that it is possible to alter the human EEG activity of alpha and beta bands when exposed to MF at frequencies corresponding to those same bands, depending on the order and period of MF conditions. This type of EEG synchronisation of driving alpha and beta EEG by alpha and beta sinusoidal MF stimulation, demonstrated in this study, could possibly be applied as therapeutic treatment(s) of particular neurophysiological abnormalities such as sleep and psychiatric disorders.

(E) Das S, Kumar S, Jain S, Avelev VD, Mathur R. Exposure to ELF- magnetic field promotes restoration of sensori-motor functions in adult rats with hemisection of thoracic spinal cord. Electromagn Biol Med. 31(3):180-194, 2012. (AS, CE, ME, BE, MA)

Clinically effective modalities of treatment for spinal cord injury (SCI) still remain unsatisfactory and are largely invasive in nature. There are reports of accelerated regeneration in injured peripheral nerves by extremely low-frequency pulsed electromagnetic field (ELF-EMF) in the rat. In the present study, the effect of (50 Hz), low-intensity (17.96 µT) magnetic field (MF) exposure of rats after-hemisection of T13 spinal cord (hSCI) was investigated on sensori-motor and locomotor functions. Rats were divided into hSCI (sham-exposed) and hSCI+MF (MF: 2 h/d X 6 weeks) groups. Besides their general conditions, locomotor function by Basso, Beattie, and Brenahan (BBB) score; motor responses to noxious stimuli by threshold of tail flick (TTF), simple vocalization (TSV), tail flick latency (TFL), and neuronal excitability by H-reflex were noted. It is found that, in the hSCI+MF group, a statistically significant improvement over the hSCI control group was noted in BBB score from post-SCI wk2 and TFL and TTF by post-hSCI wk1 and wk3, respectively. Correspondingly, TSV gradually restored by post-hSCI wk5.The threshold of H-reflex was reduced on ipsilateral side vs. contralateral side in hSCI and hSCI+MF group. A complete bladder control was dramatically restored on post-hSCI day4 (vs. day7 of hSCI group) and the survival rate was 100% in the hSCI+MF group (vs. 90%

of hSCI group). The results of our study suggest that extremely low-frequency (50 Hz), low-intensity (17.96 µT) MF exposure for 2 h/d x 6wks promotes recovery of sensori-motor behavior including locomotion and bladder control both in terms of temporal pattern and magnitude in hemisection injury of (T13) spinal cord rats.

(E) Davanipour Z, Tseng C-C, Lee PJ, Markides KS, Sobel E. Severe Cognitive Dysfunction and Occupational Extremely Low Frequency Magnetic Field Exposure among Elderly Mexican Americans. Brit J Med Med Res 4 (8): 1641-1662, 2014. (HU, BE)

Aims: This report is the first study of the possible relationship between extremely low frequency (50-60 Hz, ELF) magnetic field (MF) exposure and severe cognitive dysfunction. Earlier studies investigated the relationships between MF occupational exposure and Alzheimer's disease (AD) or dementia. These studies had mixed results, depending upon whether the diagnosis of AD or dementia was performed by experts and upon the methodology used to classify MF exposure. Study Design: Population-based case-control. Place and Duration of Study: Neurology and Preventive Medicine, Keck School of Medicine, University of Southern California, 2 years. Methodology: The study population consisted of 3050 Mexican Americans, aged 65+, enrolled in Phase 1 of the Hispanic Established Population for the Epidemiologic Study of the Elderly (H-EPESE) study. Mini-Mental State Exam (MMSE) results, primary occupational history, and other data were collected. Severe cognitive dysfunction was defined as an MMSE score below 10. The MF exposure methodology developed and used in earlier studies was used. Results: Univariate odds ratios (OR) were 3.4 (P< .03; 95% CI: 1.3-8.9) for high and 1.7 (P=.27; 95% CI: 0.7-4.1) for medium or high (M/H) MF occupations. In multivariate main effects models, the results were similar. When interaction terms were allowed in the models, the interactions between M/H or high occupational MF exposure and smoking history or age group were statistically significant, depending upon whether two (65-74, 75+) or three (65-74, 75-84, 85+) age groups were considered, respectively. When the analyses were limited to subjects aged 75+, the interactions between M/H or high MF occupations and a positive smoking history were statistically significant. Conclusion: The results of this study indicate that working in an occupation with high or M/H MF exposure may increase the risk of severe cognitive dysfunction. Smoking and older age may increase the deleterious effect of MF exposure.

(E) Del Giudice E, Facchinetti F, Nofrate V, Boccaccio P, Minelli T, Dam M, Leon A, Moschini G. Fifty Hertz electromagnetic field exposure stimulates secretion of beta-amyloid peptide in cultured human neuroglioma. Neurosci Lett. 418(1):9-12, 2007. (CS, CE, ND)

Recent epidemiological studies raise the possibility that individuals with occupational exposure to low frequency (50-60 Hz) electromagnetic fields (LF-EMF), are at increased risk of Alzheimer's disease (AD). However, the mechanisms through which LF-EMF may affect AD pathology are unknown. We here tested the hypothesis that the exposure to LF-EMF may affect amyloidogenic processes. We examined the effect of exposure to 3.1 mT 50 Hz LF-EMF on Abeta secretion in H4 neuroglioma cells stably overexpressing human mutant amyloid precursor protein. We found that overnight exposure to LF-EMF induces a significant increase of amyloid-beta peptide (Abeta) secretion, including the isoform Abeta 1-42, without affecting cell survival. These findings show for the first time that exposure to LF-EMF stimulates Abeta secretion in vitro, thus alluding to a potential link between LF-EMF exposure and APP processing in the brain.

(E) **Deng Y, Zhang Y, Jia S, Liu J, Liu Y, Xu W, Liu L. Effects of aluminum and extremely low frequency electromagnetic radiation on oxidative stress and memory in brain of mice. Biol Trace Elem Res. 156(1-3):243-252, 2013. (AS, CE, BE, OX)**

This study was aimed to investigate the effect of aluminum and extremely low-frequency magnetic fields (ELF-MF) on oxidative stress and memory of SPF Kunming mice. Sixty male SPF Kunming mice were divided randomly into four groups: control group, ELF-MF group (2 mT, 4 h/day), load aluminum group (200 mg aluminum/kg, 0.1 ml/10 g), and ELF-MF + aluminum group (2 mT, 4 h/day, 200 mg aluminum/kg). After 8 weeks of treatment, the mice of three experiment groups (ELF-MF group, load aluminum group, and ELF-MF + aluminum group) exhibited firstly the learning memory impairment, appearing that the escaping latency to the platform was prolonged and percentage in the platform quadrant was reduced in the Morris water maze (MWM) task. Secondly are the pathologic abnormalities including neuronal cell loss and overexpression of phosphorylated tau protein in the hippocampus and cerebral cortex. On the other hand, the markers of oxidative stress were determined in mice brain and serum. The results showed a statistically significant decrease in superoxide dismutase activity and increase in the levels of malondialdehyde in the ELF-MF group ($P < 0.05$ or $P < 0.01$), load aluminum group ($P < 0.01$), and ELF-MF + aluminum group ($P < 0.01$). However, the treatment with ELF-MF + aluminum induced no more damage than ELF-MF and aluminum did, respectively. In conclusion, both aluminum and ELF-MF could impact on learning memory and pro-oxidative function in Kunming mice. However, there was no evidence of any association between ELF-MF exposure with aluminum loading.

(E) **Di Loreto S, Falone S, Caracciolo V, Sebastiani P, D'Alessandro A, Mirabilio A, Zimmitti V, Amicarelli F. Fifty hertz extremely low-frequency magnetic field exposure elicits redox and trophic response in rat-cortical neurons. J Cell Physiol. 219(2):334-343, 2009. (CS, AE, CC, OX)**

Large research activity has raised around the mechanisms of interaction between extremely low-frequency magnetic fields (ELF-MFs) and biological systems. ELF-MFs may interfere with chemical reactions involving reactive oxygen species (ROS), thus facilitating oxidative damages in living cells. Cortical neurons are particularly susceptible to oxidative stressors and are also highly dependent on the specific factors and proteins governing neuronal development, activity and survival. The aim of the present work was to investigate the effects of exposures to two different 50 Hz sinusoidal ELF-MFs intensities (0.1 and 1 mT) in maturing rat cortical neurons' major anti-oxidative enzymatic and non-enzymatic cellular protection systems, membrane peroxidative damage, as well as growth factor, and cytokine expression pattern. Briefly, our results showed that ELF-MFs affected positively the cell viability and concomitantly reduced the levels of apoptotic death in rat neuronal primary cultures, with no significant effects on the main anti-oxidative defences. Interestingly, linear regression analysis suggested a positive correlation between reduced glutathione (GSH) and ROS levels in 1 mT MF-exposed cells. On this basis, our hypothesis is that GSH could play an important role in the antioxidant defence towards the ELF-MF-induced redox challenge. Moreover, the GSH-based cellular response was achieved together with a brain-derived neurotrophic factor over-expression as well as with the interleukin 1beta-dependent regulation of pro-survival signaling pathways after ELF-MF exposure.

(E) Dimitrijević D, Savić T, Anđelković M, Prolić Z, Janać B. Extremely low frequency magnetic field (50 Hz, 0.5 mT) modifies fitness components and locomotor activity of Drosophila subobscura. Int J Radiat Biol. 2014 Mar19. [Epub ahead of print] (AS, AE, DE, BE)

Purpose: Extremely low frequency (ELF) magnetic fields are essential ecological factor which may induce changes in many organisms. The aim of this study was to examine the effects in Drosophila subobscura exposed for 48 h to ELF magnetic field (50 Hz, 0.5 mT) at different developmental stages. Materials and methods: Egg-first instar larvae developmental stage of D. subobscura isofemale lines was exposed to ELF magnetic field, and fitness components (developmental time, developmental dynamics, viability and sex ratio) and locomotor activity of 3-days old males and females were monitored. Also, just eclosed D. subobscura isofemale adults were exposed to ELF magnetic field and their locomotor activity was monitored just after. Results: ELF magnetic field shortens developmental time, increases viability and does not affect sex ratio of D. subobscura. No matter which developmental stage is exposed, ELF magnetic field significantly decreases locomotor activity of adult flies, but after exposure of just eclosed adults observed change lasts longer. Conclusions: Applied ELF magnetic field modifies fitness components and locomotor activity of D. subobscura. Observed effects can be attributed to the influence of magnetic field on different stages of development where the hormonal and nervous systems play important role in the control of examined parameters.

(E) Duan Y, Wang Z, Zhang H, He Y, Lu R, Zhang R, Sun G, Sun X. The preventive effect of lotus seedpod procyanidins on cognitive impairment and oxidative damage induced by extremely low frequency electromagnetic field exposure. Food Funct. 4(8):1252-1262, 2013. (AS, CE, BE, OX)

The present study investigated the effects of lotus seedpod procyanidins (LSPCs) administered by oral gavage on the cognitive deficits and oxidative damage of mice at extremely low frequency electromagnetic field (ELF-EMF) exposure (50 Hz, 8 mT, 28 days). The results showed that 90 mg kg^{-1} LSPCs treatment significantly increased body weight compared with the ELF-EMF group at ELF-EMF exposure and effectively maintained liver index, thymus index, kidney index and spleen index close to normal. A water maze test indicated that learning and memory abilities of the ELF-EMF group deteriorated significantly with ELF-EMF exposure when compared with the control group, but the ELF-EMF + LSPCs90 group had remarkably improved learning and memory abilities compared with the ELF-EMF group. Malondialdehyde (MDA), reactive oxygen species (ROS), nitric oxide (NO) and nitric oxide synthase (NOS) mostly exhibited significant increases, while the activities of glutathione peroxidase (GPx), catalase (CAT) and superoxide dismutase (SOD) decreased significantly under ELF-EMF exposure in the ELF-EMF group. LSPCs (especially 60, 90 mg kg^{-1}) administration decreased MDA, ROS, NO content and lowered NOS activity in LSPCs treatment groups. Furthermore, LSPCs (60, 90 mg kg^{-1}) treatment significantly augmented GPx, CAT, SOD activity in the hippocampus and serum. Pathological observation showed that number of pyramidal cells of the CA1 and CA3 regions of the hippocampus of the LSPCs treatment groups was significantly greater than the ELF-EMF group. All the data suggested that the LSPCs can effectively prevent learning and memory damage and oxidative damage caused by the ELF-EMF, most likely through the ability of LSPCs to scavenge oxygen free radicals and to stimulate antioxidant enzyme activity.

(E) El Gohary MI, Salama AA, El Saeid AA, El Sayed TM, Kotb HS. Influence of Magnetic Field on Brain Activity During Administration of Caffeine. Cell Biochem Biophys. 67(3):929-933, 2013. (AS, CE, EE)

The aim of the present work is to evaluate the effect of caffeine, the world's most popular psychoactive drug, on the electric activity of the rat's brain that exposed to extremely low-frequency magnetic field (ELF-MF), during 15 days. The obtained results showed that administration of caffeine in a group of rats by dose of 10 mg/kg (equivalent to human daily consumption) caused a reduction in the mean power amplitude of electroencephalogram (EEG) trace for almost all frequency bands especially α (8-12 Hz). It was observed that the influence of caffeine was more evident in motor cortex than in visual cortex. While the exposure of another group to ELF-MF of intensity 0.2 mT during the same period caused an enhancement in the mean power amplitude of most EEG frequency bands; this was more observed in the right hemisphere of the brain than that of the left hemisphere. The administration of caffeine while rats were exposed to ELF-MF, led, after 5 days of exposure, to a great increase in the mean power amplitude of α band at all places of recording electrodes. It may be concluded that caffeine administration was more effective in reducing the hazardous of ELF-MF in motor cortex than in visual cortex.

(E) Esmekaya MA, Acar SI, Kıran F, Canseven AG, Osmanagaoglu O, Seyhan N. Effects of ELF magnetic field in combination with Iron(III) chloride (FeCl3) on cellular growth and surface morphology of Escherichia coli (E. coli). Appl Biochem Biotechnol. 169(8):2341-2349, 2013. (CS, AE, MC)

This study investigated the effects of extremely low frequency (ELF) magnetic field with/without iron(III) chloride (FeCl3) on bacterial growth and morphology. The ELF exposures were carried out using a pair of Helmholtz coil-based ELF exposure system which was designed to generate 50 Hz sinusoidal magnetic field. The field was approximately uniform throughout the axis of the coil pair. The samples which were treated or non-treated with different concentrations FeCl3 were exposed to 50 Hz, 2 millitesla (mT) magnetic field for 24 h. ELF effect on viability was assessed in terms of viable colony counts (in colony-forming unit per milliliter) with the standard plate count technique. Scanning electron microscopy was used to investigate the magnetic field effect on surface morphology of Escherichia coli. No significant results were seen in terms of cell viability between ELF and sham-exposed bacterial strains. Similarly, FeCl3 treatment did not change cell viability of E. coli samples. However, we observed some morphological changes on E. coli cell surfaces. Pore formations and membrane destruction were seen on the surface of 24 h ELF field-exposed cells. We concluded that ELF magnetic field exposure at 2 mT does not affect cell viability; however, it may affect bacterial surface morphology.

(E) Falone S, Mirabilio A, Carbone MC, Zimmitti V, Di Loreto S, Mariggiò MA, Mancinelli R, Di Ilio C, Amicarelli F. Chronic exposure to 50Hz magnetic fields causes a significant weakening of antioxidant defence systems in aged rat brain. Int J Biochem Cell Biol. 40(12):2762-2770, 2008. (AS, CE, CC, OX)

Several studies suggest that extremely low-frequency magnetic fields (ELF-MFs) may enhance the free radical endogenous production. It is also well known that one of the unavoidable consequences of ageing is an overall oxidative stress-based decline in several physiological functions and in the general resistance to stressors. On the basis of these assumptions, the aim of this study was to establish whether the ageing process can increase susceptibility towards widely present ELF-MF-mediated pro-oxidative challenges. To this end, female Sprague-Dawley rats were continuously exposed to a sinusoidal 50 Hz, 0.1 mT magnetic field for 10 days. Treatment-induced changes in the major antioxidant protection systems and in the neurotrophic support were investigated, as a function of the age of the subjects. All analyses were performed in brain cortices, due to the high susceptibility of neuronal cells to oxidative injury. Our results indicated that ELF-MF exposure significantly affects anti-oxidative capability, both in young and aged animals, although in opposite ways. Indeed, exposed young individuals enhanced their neurotrophic signalling and anti-oxidative enzymatic defence against a possible ELF-MF-mediated increase in oxygen radical species. In contrast, aged subjects were not capable of increasing their defences in response to ELF-MF treatment but, on the contrary, they underwent a significant decrease in the major antioxidant enzymatic activities. In conclusion, our data seem to suggest that the exposure to ELF-MFs may act as a risk factor for the occurrence of oxidative stress-based nervous system pathologies associated with ageing.

(E) Fournier NM, Mach QH, Whissell PD, Persinger MA. Neurodevelopmental anomalies of the hippocampus in rats exposed to weak intensity complex magnetic fields throughout gestation. Int J Dev Neurosci. 2012 Jul 31. [Epub ahead of print] (AS, CE, DE, BE, MC)

There has been increasing interest on the possible harmful effects of prenatal exposure to magnetic fields. To investigate the effect of weak intensity magnetic fields on the prenatal brain, pregnant Wistar rats were continuously exposed to one of four intensities (reference: 5-20nT; low 30-50nT; medium 90-580nT; high 590-1200nT) of a complex magnetic field sequence designed to interfere with brain development. As adults, rats exposed to the low-intensity (30-50nT) complex magnetic field displayed impairments in contextual fear learning and showed anomalies in the cytological and morphological development of the hippocampus. In particular, low-intensity exposures resulted in a reduction in overall hippocampal size and promoted subtle dysgenesis of the CA1 and CA3 regions. In contrast, exposure to weaker or stronger intensities of the same complex magnetic field pattern did not interfere with hippocampal development or fear behavior. These findings suggest that prenatal exposure to complex magnetic fields of a narrow intensity window during development can result in subtle but permanent alterations in hippocampal microstructure and function that can have lasting effects on behavior.

(E) Frilot C 2nd, Carrubba S, Marino AA. Transient and steady-state magnetic fields induce increased fluorodeoxyglucose uptake in the rat hindbrain. Synapse. 65(7):617-623, 2011. (HU, AE, CC)

We inquired into the biophysical basis of the ability of weak electromagnetic fields (EMFs) to trigger onset and offset evoked potentials, and to produce steady-state changes in the electroencephalogram (EEG). Rats were exposed to a 2.5-G, 60-Hz magnetic field and the neuroanatomical region of glucose activation associated with the effect of the field on the EEG was identified by positron emission tomography (PET) using fluorodeoxyglucose (FDG). Paired emission scans from the same animal with and without field treatment were differenced and averaged, and t values of the brain voxels computed using the pooled standard deviation were compared with a calculated critical t value to identify the field-activated voxels. Increased glucose utilization occurred in hindbrain voxels when the field was applied orthogonally to the sagittal plane, but not when the angle between the field and the sagittal plane varied randomly. Distinct FDG activation effects were observed in response to transient (both onset and offset) and steady-state magnetic stimuli. Observations of increased glucose utilization induced by magnetic stimuli and its dependence on the direction of the field suggested that signal transduction was mediated by a force detector and that the process and/or early post-transduction processing occurred in the hindbrain.

(E) Fu Y, Wang C, Wang J, Lei Y, Ma Y. Long-term exposure to extremely low-frequency magnetic fields impairs spatial recognition memory in mice. Clin Exp Pharmacol Physiol. 35(7):797-800, 2008. (AS, CE, BE)

In the present study, we investigated the short- and long-term effects of extremely low-frequency (ELF) magnetic fields on spatial recognition memory in mice by using a two-trial recognition Y-maze that is based on the innate tendency of rodents to explore novel environments. 2. Mice were exposed to 25 or 50 Hz electromagnetic fields for either 7 (short term) or 25 days (long term) and then tested in the Y-maze. 3. The results indicated that neither short- nor long-term exposure to magnetic fields affected the locomotor activity of mice in the Y-maze. However, long-term exposure to 50 Hz fields reduced recognition of the novel arm. 4. Our findings suggest that ELF magnetic fields impair spatial recognition memory in the Y-maze depending on the field strength and/or duration of exposure.

(NE) Gavoçi E, Zironi I, Remondini D, Virelli A, Castellani G, Del Re B, Giorgi G, Aicardi G, Bersani F. ELF magnetic fields tuned to ion parametric resonance conditions do not affect TEA-sensitive voltage-dependent outward K(+) currents in a human neural cell line. Bioelectromagnetics. 34(8):579-88, 2013. (CS, AE, CC)

Despite the experimental evidence of significant biological effects of extremely low frequency (ELF) magnetic fields (MFs), the underlying mechanisms are still unclear. Among the few mechanisms proposed, of particular interest is the so called "ion parametric resonance (IPR)" hypothesis, frequently referred to as theoretical support for medical applications. We studied the effect of different combinations of static (DC) and alternating (AC) ELF MFs tuned on resonance conditions for potassium (K(+)) on TEA-sensitive voltage-dependent outward K(+) currents in the human neuroblastoma BE(2)C cell line. Currents through the cell membrane were measured by whole-cell patch clamp before, during, and after exposure to MF. No significant changes in K(+) current density were found. This study does not confirm the IPR hypothesis at the level of TEA-sensitive voltage-dependent outward K(+) currents in our experimental conditions. However, this is not a direct disprove of the hypothesis, which should be investigated on other ion channels and at single channel levels also.

(NE) Glover PM, Eldeghaidy S, Mistry TR, Gowland PA. Measurement of visual evoked potential during and after periods of pulsed magnetic field exposure. J Magn Reson Imaging. 26(5):1353-1356, 2007. (HU, EE)

PURPOSE: To study the effect of switched magnetic fields used in MR scanners on the visual evoked potential (VEP) in human subjects. MATERIALS AND METHODS: We have used an MRI gradient coil, remote from an MRI magnet to produce a time-varying magnetic field (0.5 kHz, peak field approximately 8.7 T/second) in the human brain without the confounding effects of static field exposure or accompanying acoustic noise. The VEP response to a 2-Hz reversal, 8 x 8 checkerboard, occupying 20 degrees of the visual field was recorded from occipital locations O1 and O2. VEP recordings were made every five minutes before, during, and after a 10-minute magnetic field exposure period for seven subjects. RESULTS: In contradiction to studies previously reported in the literature for fields of 50 Hz and 60 mT, no significant effects on the peak amplitude or latency of the VEP P100 O1 and O2 responses were found. CONCLUSION: Switched magnetic fields of a level and frequency comparable to those used in MRI do not have a significant effect on primary retinal or visual processing.

(E) Gulturk S, Demirkazik A, Kosar I, Cetin A, Dökmetas HS, Demir T. Effect of exposure to 50 Hz magnetic field with or without insulin on blood-brain barrier permeability in streptozotocin-induced diabetic rats. Bioelectromagnetics. 31(4):262-269, 2010. (AS, CE, ME)

We investigated the effect of long-term exposure to modulation magnetic field (MF), insulin, and their combination on blood-brain barrier (BBB) permeability in a diabetic rat model. Fifty-three rats were randomly assigned to one of six groups: sham, exposed to no MF; MF, exposed to MF; diabetes mellitus (DM), DM induced with streptozotocin (STZ); DM plus MF (DMMF); DM plus insulin therapy (DMI); and DM plus insulin therapy plus MF (DMIMF). All the rats underwent Evans blue (EB) measurement to evaluate the BBB 30 days after the beginning of experiments. The rats in MF, DMMF, and DMIMF groups were exposed to MF (B = 5 mT) for 165 min every day for 30 days. Mean arterial blood pressure (MABP), body mass, and serum glucose level of the study rats were recorded. The extravasation of brain EB of the MF, DM, DMMF, DMI, and DMIMF groups was higher than that of the sham group and the extravasation of right hemisphere of the DMIMF group was highest ($P < 0.05$). The post-procedure body mass of the sham and MF groups were significantly higher than those of the DM and DMMF groups ($P < 0.05$). In the DM, DMMF, DMI, and DMIMF groups, the baseline glucose was significantly lower than the post-procedure glucose ($P < 0.05$). DM and MF increase BBB permeability; in combination, they cause more increase in BBB permeability, and insulin decreases their effect on BBB. Improved glucose metabolism may prevent body mass loss and the hypoglycemic effect of MF. DM increases MABP but MF causes no additional effect.

(E) Gutiérrez-Mercado YK, Cañedo-Dorantes L, Gómez-Pinedo U, Serrano-Luna G, Bañuelos-Pineda J, Feria-Velasco A. Increased vascular permeability in the circumventricular organs of adult rat brain due to stimulation by extremely low frequency magnetic fields. Bioelectromagnetics. 34(2):145-155, 2013. (AS, CE, MC)

It has been demonstrated that the exposure of biological systems to magnetic fields (MFs) can produce several beneficial effects: tissue recovery in chronic wounds, re-establishment of blood circulation after tissue ischemia or in necrotic tissues, improvement after epileptic episodes, angiogenesis, etc. In the current study, the effects of extremely low frequency (ELF) MF on the capillaries of some circumventricular organs (CVOs) are demonstrated; a vasodilator effect is reported as well as an increase in their permeability to non-liposoluble substances. For this study, 96 Wistar male rats (250 g body mass) were used and divided into three groups of 32 rats each: a control group (no treatment); a sham ELF-MF group; and an experimental group subjected to ELF-MF (120 Hz harmonic waves and 0.66 mT, root mean square) by the use of Helmholtz coils. All animals were administered colloidal carbon (CC) intravenously to study, through optical and transmission electron microscopy, the capillary permeability in CVOs and the blood-brain barrier (BBB) in brain areas. An increase in capillary permeability to CC was detected in the ELF-MF-exposed group as well as a significant increase in vascular area (capillary vasodilation); none of these effects were observed in individuals of the control and sham ELF-MF groups. It is important to investigate the mechanisms involved in the phenomena reported here in order to explain the effects of ELF-MF on brain vasculature.

(E) Harakawa S, Nedachi T, Hori T, Takahashi K, Tochio K, Inoue N. Effect of electric field in conditioned aversion response. J Vet Med Sci. 70(6):611-613, 2008. (AS, AE, BE, EF)

The aim of the present study was to estimate whether rat sense exogenous electric field (EF) including one used in our previous studies. Employing a conditioned place aversion response paradigm based on an aversive behavior against light environment, alteration in both voluntary behavior of Wistar rat to a 50 Hz sinusoidal EF was examined. Following conditioning without EF, the times spent in white place in rats was significantly shortened (P<0.05). While, such changes were not shown in rats conditioned with EF. Thus, it was considered that the aversion response to light environment was interfered by exposure to EF. An interference in recognition of brightness via EF induced effect to visual system or in learning system via direct effect to central nerve system was considerable as a factor for EF-induced effect. In addition, it was remained that rat possibly sense exposure to EF as preferable. In order to confirm which factor functioned, further studies are needed.

(E) He LH, Shi HM, Liu TT, Xu YC, Ye KP, Wang S. Effects of extremely low frequency magnetic field on anxiety level and spatial memory of adult rats. Chin Med J (Engl). 124(20):3362-3366, 2011. (AS, CE, BE)

BACKGROUND: As the widespread use of electric devices in modern life, human are exposed to extremely low frequency magnetic fields (ELF MF) much more frequently than ever. Over the past decades, a substantial number of epidemiological and experimental studies have demonstrated that ELF MF (50 Hz) exposure is associated with increased risk of various health effects. The present study examined the effects of chronic exposure to ELF MF on anxiety level and spatial memory of adult rats. METHODS: The 50-Hz ELF MF was used during the whole experimental procedures and the value of magnetic field (MF) was set to 2 mT. Adult rats were divided randomly to control, MF 1 hour and MF 4 hours group. Anxiety-related behaviors were examined in the open field test and the elevated plus maze; changes in spatial learning and memory were determined in Morris water maze after 4 weeks of daily exposure. RESULTS:

Rats in MF 4 hours group had increased anxiety-like behaviors with unaltered locomotor activity. In the Morris water maze test, rats had reduced latency to find the hidden platform and improved long-term memory of former location of platform without changes in short-term memory and locomotor activity. CONCLUSION: Chronic ELF MF exposure has anxiogenic effect on rats, and the promoting effects on spatial learning and long-term retention of spatial memory.

(E) He YL, Liu DD, Fang YJ, Zhan XQ, Yao JJ, Mei YA. Exposure to extremely low-frequency electromagnetic fields modulates Na+ currents in rat cerebellar granule cells through increase of AA/PGE2 and EP receptor-mediated cAMP/PKA pathway. PLoS One. 2013;8(1):e54376. doi: 10.1371/journal.pone.0054376. (CS, AE, CC, EE)

Although the modulation of Ca(2+) channel activity by extremely low-frequency electromagnetic fields (ELF-EMF) has been studied previously, few reports have addressed the effects of such fields on the activity of voltage-activated Na(+) channels (Na(v)). Here, we investigated the effects of ELF-EMF on Na(v) activity in rat cerebellar granule cells (GCs). Our results reveal that exposing cerebellar GCs to ELF-EMF for 10-60 min significantly increased Na(v) currents (I(Na)) by 30-125% in a time- and intensity-dependent manner. The Na(v) channel steady-state activation curve, but not the steady-state inactivation curve, was significantly shifted (by 5.2 mV) towards hyperpolarization by ELF-EMF stimulation. This phenomenon is similar to the effect of intracellular application of arachidonic acid (AA) and prostaglandin E(2) (PGE(2)) on I(Na) in cerebellar GCs. Increases in intracellular AA, PGE(2) and phosphorylated PKA levels in cerebellar GCs were observed following ELF-EMF exposure. Western blottings indicated that the Na(V) 1.2 protein on the cerebellar GCs membrane was increased, the total expression levels of Na(V) 1.2 protein were not affected after exposure to ELF-EMF. Cyclooxygenase inhibitors and PGE(2) receptor (EP) antagonists were able to eliminate this ELF-EMF-induced increase in phosphorylated PKA and I(Na). In addition, ELF-EMF exposure significantly enhanced the activity of PLA(2) in cerebellar GCs but did not affect COX-1 or COX-2 activity. Together, these data demonstrate for the first time that neuronal I(Na) is significantly increased by ELF-EMF exposure via a cPLA2 AA PGE(2) EP receptors PKA signaling pathway.

(E) Hung CS, Anderson C, Horne JA, McEvoy P. Mobile phone 'talk-mode' signal delays EEG-determined sleep onset. Neurosci Lett. 421(1):82-86, 2007. (HU, AE, EE, BE)

Mobile phones signals are pulse-modulated microwaves, and EEG studies suggest that the extremely low-frequency (ELF) pulse modulation has sleep effects. However, 'talk', 'listen' and 'standby' modes differ in the ELF (2, 8, and 217Hz) spectral components and specific absorption rates, but no sleep study has differentiated these modes. We used a GSM900 mobile phone controlled by a base-station simulator and a test SIM card to simulate these three specific modes, transmitted at 12.5% (23dBm) of maximum power. At weekly intervals, 10 healthy young adults, sleep restricted to 6h, were randomly and single-blind exposed to one of: talk, listen, standby and sham (nil signal) modes, for 30 min, at 13:30 h, whilst lying in a sound-proof, lit bedroom, with a thermally insulated silent phone beside the right ear. Bipolar EEGs were recorded continuously, and subjective ratings of sleepiness obtained every 3 min (before, during and after exposure). After exposure the phone and base-station were switched off, the bedroom darkened, and a 90 min sleep opportunity followed. We report on sleep onset using: (i) visually scored latency to onset of stage 2 sleep, (ii) EEG power spectral analysis. There was no condition effect for subjective sleepiness. Post-exposure, sleep latency after talk mode was markedly and significantly delayed beyond listen and sham modes. This condition effect over time was also quite evident in 1-4Hz EEG frontal power, which is a frequency range particularly sensitive to sleep onset. It is possible that 2, 8, 217Hz modulation may differentially affect sleep onset.

(E) Ishay JS, Plotkin M, Volynchik S, Shaked M, Schuss Z, Bergman DJ. Exposure to an additional alternating magnetic field affects comb building by worker hornets. Physiol Chem Phys Med NMR. 39(1):83-88, 2007. (AS, CE, BE)

Oriental hornet workers, kept in an Artificial Breeding Box (ABB) without a queen, construct within a few days brood combs of hexagonal cells with apertures facing down. These combs possess stems that fasten the former to the roof of the ABB. In an ABB with adult workers (more than 24 h after eclosion), exposed to an AC (50 Hz) magnetic field of a magnitude of B = 50-70 mGauss, the combs and cells are built differently from those of a control ABB, subjected only to the natural terrestrial magnetic field. The effects of the additional magnetic field consist of (a) 35-55% smaller number of cells and fewer eggs in each comb, (b) disrupted symmetry of building, with many deformed and imperfectly hexagonal cells, and (c) more delicate and slender comb stems.

(E) Jadidi M, Firoozabadi SM, Rashidy-Pour A, Sajadi AA, Sadeghi H, Taherian AA. Acute exposure to a 50 Hz magnetic field impairs consolidation of spatial memory in rats. Neurobiol Learn Mem. 88(4):387-392, 2007. (AS, CE, BE)

This study was planned to evaluate the effect of an exposure to magnetic fields on consolidation and retrieval of hippocampus dependent spatial memory using a water maze. In Experiments 1 and 2, rats were trained in a hidden version (spatial) of water maze task with two blocks of four trials. The retention of spatial memory was evaluated 48 h later. Exposure to a 50 Hz 8 mT, but not 2 mT magnetic fields for 20 min immediately after training impaired retention performance. The same time exposure shortly before retention testing had no effect. In Experiment 3, rats were trained in a cued version of water maze with two blocks of four trials. Exposure to magnetic field at 8 mT for 20 min immediately after training did not impair retention performance. These findings indicate that acute exposure to a 50 Hz magnetic field at 8 mT for short time can impair consolidation of spatial memory.

(E) Janać B, Tovilović G, Tomić M, Prolić Z, Radenović L. Effect of continuous exposure to alternating magnetic field (50 Hz, 0.5 mT) on serotonin and dopamine receptors activity in rat brain. Gen Physiol Biophys. 28 Spec No:41-46, 2009. (AS, CE, FC)

External magnetic fields (MFs) have the ability to modify motor activity of animals, complex type of behaviour connected with dopaminergic and serotonergic neurotransmissions in the brain. Thus, the purpose of this study was to examine MF-induced changes in the activity of serotonin 5-HT(2A) receptors in the prefrontal cortex, as well as dopamine D(1) and D(2) receptors in the striatum of adult Wistar rats, considering their involvement in motor behavior regulation. Experimental animals were continuously exposed to extremely low frequency MF (ELF-MF, 50 Hz, 0.5 mT) for 1, 3, and 7 days. Subsequently, binding properties (K(d) and

B(max)) of receptors were determined by in vitro radioligand receptor binding assays. It was shown that the affinity of serotonin 5-HT(2A) receptors decreased and their density increased in the prefrontal cortex of rats after ELF-MF exposure. Regarding affinity, this effect was duration-dependent and most prominent after 7-day of ELF-MF exposure. In contrast to serotonin 5-HT(2A) receptors in the prefrontal cortex, ELF-MF had no significant effect on the affinity and density of dopamine D(1) and D(2) receptors in the striatum. We can conclude that continuous exposure to ELF-MF up to 7 days affects cortical serotonergic neurotransmission, whereby intensity of these changes depends on ELF-MF exposure duration.

(E) Janać B, Selaković V, Rauš S, Radenović L, Zrnić M, Prolić Z. Temporal patterns of extremely low frequency magnetic field-induced motor behavior changes in Mongolian gerbils of different age. Int J Radiat Biol. 88(4):359-366, 2012. (AS, CE, BE)

PURPOSE: The aim of this study was to investigate the influence of extremely low frequency magnetic field (ELF-MF) on different behavior parameters (locomotion, stereotypy, and immobility) in 3- and 10-month-old male Mongolian gerbils. MATERIALS AND METHODS: The animals were continuously exposed to ELF-MF (50 Hz; 0.1, 0.25 and 0.5 mT) for seven days. Their behavior was monitored for 60 min in the open field after the 1st, 2nd, 4th, and 7th day of exposure (immediate effect), and three days after ELF-MF exposure had been ceased (delayed effect). RESULTS: In 3-month-old gerbils, exposure to ELF-MF (0.1, 0.25 and 0.5 mT) increased motor behavior (locomotion and stereotypy), and consequently decreased immobility. Additionally, ELF-MF had delayed effect (except 0.25 mT) on stereotypy and immobility. In 10-month-old gerbils, ELF-MF of 0.1, 0.25 and 0.5 mT induced decrease, slight increase, and pronounced stimulation of motor behavior, respectively. Regardless of magnetic induction value, increased motor behavior was observed three days after ELF-MF exposure has been ceased (delayed effect). CONCLUSIONS: It can be proposed that the specific temporal patterns of ELF-MF-induced motor behavior changes in 3- and 10-month-old gerbils are a consequence of age-dependent morpho-functional differences in the brain structures responsible for a control of motor behavior.

(E) Kim HJ, Jung J, Park JH, Kim JH, Ko KN, Kim CW. Extremely low-frequency electromagnetic fields induce neural differentiation in bone marrow derived mesenchymal stem cells. Exp Biol Med (Maywood). 238(8):923-931, 2013. (CS, AE, MC, MA)

Extremely low-frequency electromagnetic fields (ELF-EMF) affect numerous biological functions such as gene expression, cell fate determination and even cell differentiation. To investigate the correlation between ELF-EMF exposure and differentiation, bone marrow derived mesenchymal stem cells (BM-MSCs) were subjected to a 50-Hz electromagnetic field during in vitro expansion. The influence of ELF-EMF on BM-MSCs was analysed by a range of different analytical methods to understand its role in the enhancement of neural differentiation. ELF-EMF exposure significantly decreased the rate of proliferation, which in turn caused an increase in neuronal differentiation. The ELF-EMF-treated cells showed increased levels of neuronal differentiation marker (MAP2), while early neuronal marker (Nestin) was down-regulated. In addition, eight differentially expressed proteins were detected in two-dimensional electrophoresis maps, and were identified using ESI-Q-TOF LC/MS/MS. Among them, ferritin light chain, thioredoxin-dependent peroxide reductase, and tubulin β-6 chain were up-regulated in the ELF-EMF-stimulated group. Ferritin and thioredoxin-dependent peroxide reductase are involved in a wide variety of functions, including Ca(2+) regulation, which is a critical component of neurodegeneration. We also observed that the intracellular Ca(2+) content was significantly elevated after ELF-EMF exposure, which strengthens the modulatory role of ferritin and thioredoxin-dependent peroxide reductase, during differentiation. Notably, western blot analysis indicated significantly increased expression of the ferritin light chain in the ELF-EMF-stimulated group (0.60 vs. 1.08; $P < 0.01$). These proteins may help understand the effect of ELF-EMF stimulation on BM-MSCs during neural differentiation and its potential use as a clinically therapeutic option for treating neurodegenerative diseases.

(E) Kitaoka K, Kitamura M, Aoi S, Shimizu N, Yoshizaki K. Chronic exposure to an extremely low-frequency magnetic field induces depression-like behavior and corticosterone secretion without enhancement of the hypothalamic-pituitary-adrenal axis in mice. Bioelectromagnetics. 34(1):43-51, 2013. (AS, CE, BE, CC)

An extremely low-frequency magnetic field (ELF-MF) is generated by power lines and household electrical devices. Many studies have suggested an association between chronic ELF-MF exposure and anxiety and/or depression. The mechanism of these effects is assumed to be a stress response induced by ELF-MF exposure. However, this mechanism remains controversial. In the present study, we investigated whether chronic ELF-MF exposure (intensity, 3 mT; total exposure, 200 h) affected emotional behavior and corticosterone synthesis in mice. ELF-MF-treated mice showed a significant increase in total immobility time in a forced swim test and showed latency to enter the light box in a light-dark transition test, compared with sham-treated (control) mice. Corticosterone secretion was significantly high in the ELF-MF-exposed mice; however, no changes were observed in the amount of the adrenocorticotropic hormone and the expression of genes related to stress response. Quantification of the mRNA levels of adrenal corticosteroid synthesis enzymes revealed a significant reduction in Cyp17a1 mRNA in the ELF-MF-exposed mice. Our findings suggest the possibility that high intensity and chronic exposure to ELF-MF induces an increase in corticosterone secretion, along with depression- and/or anxiety-like behavior, without enhancement of the hypothalamic-pituitary-adrenal axis.

(E) Korpinar MA, Kalkan MT, Tuncel H. The 50 Hz (10 mT) sinusoidal magnetic field: effects on stress-related behavior of rats. Bratisl Lek Listy. 113(9):521-524, 2012. (AS, CE, BE)

Purpose: The purpose of this study was to investigate the behavioral changes induced by 50 Hz, 10 mT flux density Sinusoidal Magnetic Field (MF). Material and methods: Seventy-six young adult male Wistar albino rats were used in the study. They were separated into two groups: control group (C) n=38; MF group n=38. C animals were left under the same conditions with the MF group for 21 days but with prevented or avoided exposure to MF. Anxiety and stress-related behavioral changes were investigated by elevated plus-maze and hole-board systems. Just before being tested in the maze, each animal was tested by means of the hole-board method in order to separate the directed exploration behavior and locomotion activity changes from anxiety-related behavior. Results: In the hole-board system parameters there were no statistically significant differences between the two groups. There was a statistically significant difference between MF and C groups when the ratio of time spent on open arms to the total time spent on all arms was evaluated (0.12±0.08 and 0.34±0.18 respectively and p <0.01). Conclusion: Our results suggest that after 21 days, a continuous exposure to extremely low frequency of magnetic field (50 Hz, 10 mT) has no significant effect on activity and exploration activity but significantly induces stress and anxiety-related behavior in rats (Tab. 2, Fig. 9, Ref. 19).

(E) Kumar S, Jain S, Behari J, Avelev VD, Mathur R. Effect of magnetic field on food and water intake and body weight of spinal cord injured rats. Indian J Exp Biol. 48(10):982-986, 2010. (AS, CE, MA)

Chronic (2 h/d x 8 weeks) exposure to magnetic field (MF; 50 Hz, 17.9 microT) in complete spinal cord (T13) transected rats restored food intake (FI), water intake (WI) and body weight (BW) which were decreased in the spinal cord injured rats. The results suggest a significant beneficial effect of chronic exposure to magnetic field of paraplegic rats.

(E) Kumar S, Jain S, Velpandian T, Petrovich Gerasimenko Y, D Avelev V, Behari J, Behari M, Mathur R. Exposure to extremely low-frequency magnetic field restores spinal cord injury-induced tonic pain and its related neurotransmitter concentration in the brain. Electromagn Biol Med. 32(4):471-483, 2013. (AS, CE, BE, CC, MA)

Spinal cord injury (SCI) is unequivocally reported to produce hyperalgesia to phasic stimuli, while both hyper- and hypoalgesia to tonic stimuli. The former is spinally mediated and the latter centrally. Besides, its management is unsatisfactory. We report the effect of magnetic field (MF; 17.96 μT, 50 Hz) on tonic pain behavior and related neurotransmitters in the brain of complete thoracic (T13) SCI rats at week 8. Adult male Wistar rats were divided into Sham, SCI and SCI+MF groups. Formalin-pain behavior was compared utilizing 5 min block pain rating (PR), 60 min session-PR, time spent in various categories of increasing pain (T0-T3) and flinch incidences. Serotonin (5-HT), dopamine (DA), norepinepherine (NE), gamma-aminobutyric acid (GABA), glutamate and glycine were estimated in brain tissue by liquid chromatography-mass spectrometry. Session-PR, block-PR and number of flinches were significantly lower, while time spent in categories 0-1 was higher in the SCI versus Sham group. These parameters were comparable in the SCI+MF versus Sham group. 5-HT concentration in cortex, remaining forebrain areas and brain stem (BS), was lower while GABA and NE were higher in BS of SCI, which were comparable with Sham in the SCI+MF group. The concentration of DA, glutamate and glycine was comparable amongst the groups. The data indicate significant hypoalgesia in formalin pain while increased in GABA, NE and decreased in 5-HT post-SCI, which were restored in the SCI+MF group. We suggest beneficial effect of chronic (2 h/day × 8 weeks) exposure to MF (50 Hz, 17.96 μT) on tonic pain that is mediated by 5-HT, GABA and NE in complete SCI rats.

(E) Lahijani MS, Bigdeli MR, Kalantary S. Effects of sinusoidal electromagnetic fields on histopathology and structures of brains of preincubated white Leghorn chicken embryos. Electromagn Biol Med. 30(3):146-157, 2011. (AS, AE, MC, DE)

There are several reports indicating linkages between exposures to 50-60 Hz electromagnetic fields and abnormalities in the early stages of chicken embryonic development. Based on our previous published research carried out at the Department of Animal Sciences, Faculty of

Biological Sciences, Shahid Beheshti University, effects of sinusoidal electromagnetic fields on histopathology and structures of brains of preincubated white leghorn hen eggs were investigated. Three hundred healthy fresh fertilized eggs (55-65 gr) were divided into three groups of experimental (n = 50), control (n = 75), and sham (n = 75). Experimental eggs (inside the coil) were exposed to 3 different intensities of 1.33, 2.66, and 7.32 mT and sham groups were located inside the same coil with no exposure, for 24 h before incubation. Control, sham, and experimental groups were all incubated in an incubator (38 ± 0.5(°)C, 60% humidity) for 14 days. 14-day old chicken embryos were removed by C-sections, and the brains of all embryos of all groups were fixed in formalin(10%), stained with H&E and TUNEL assay, for studying the histopatholog and process of apoptosis. The brains of other embryos were prepared for Scanning Electeron Microscope. Results showed electromagnetic fields have toxic effects on brain cells by increasing the number of apoptotic cells and degeneration of brains' tissues of exposed chicken embryos. These findings suggest that the electromagnetic fields induce brain damages at different levels.

(E) Legros A, Corbacio M, Beuter A, Modolo J, Goulet D, Prato FS, Thomas AW. Neurophysiological and behavioral effects of a 60 Hz, 1,800 µT magnetic field in humans. Eur J Appl Physiol. 112(5):1751-1762, 2012. (HU, AE, BE)

The effects of time-varying magnetic fields (MF) on humans have been actively investigated for the past three decades. One important unanswered question is the potential for MF exposure to have acute effects on human biology. Different strategies have been used to tackle this question using various physiological, neurophysiological and behavioral indicators. For example, researchers investigating electroencephalography (EEG) have reported that extremely low frequency (ELF, <300 Hz) MF can increase resting occipital alpha rhythm (8-12 Hz). Interestingly, other studies have demonstrated that human motricity can be modulated by ELF MF: a reduction of anteroposterior standing balance or a decrease of physiological tremor intensity have been reported as consequences of exposure. However, the main limitation in this domain lies in the lack of results replication, possibly originating from the large variety of experimental approaches employed. Therefore, the present study aimed to investigate the effects of a 60 Hz, 1,800 µT MF exposure on neurophysiological (EEG) and neuromotor (standing balance, voluntary motor function, and physiological tremor) aspects in humans using a single experimental procedure. Though results from this study suggest a reduction of human standing balance with MF exposure, as well as an increase of physiological tremor amplitude within the frequency range associated with central nervous system contribution, no exposure effect appeared on other investigated parameters (e.g., EEG or voluntary motor control). These results suggest that 1 h of 60 Hz, 1,800 µT MF exposure may modulate human involuntary motor control without being detected in the cortical electrical activity.

(E) Leone L, Fusco S, Mastrodonato A, Piacentini R, Barbati SA, Zaffina S, Pani G, Podda MV, Grassi C. Epigenetic Modulation of Adult Hippocampal Neurogenesis by Extremely Low-Frequency Electromagnetic Fields. Mol Neurobiol. 2014 Feb 16. [Epub ahead of print] (AS, CS, CE, AE, BE, CC, MA)

Throughout life, adult neurogenesis generates new neurons in the dentate gyrus of hippocampus that have a critical role in memory formation. Strategies able to stimulate this endogenous process have raised considerable interest because of their potential use to treat neurological disorders entailing cognitive impairment. We previously reported that mice exposed to extremely low-frequency electromagnetic fields (ELFEFs) showed increased hippocampal neurogenesis. Here, we demonstrate that the ELFEF-dependent enhancement of hippocampal neurogenesis improves spatial learning and memory. To gain insights on the molecular mechanisms underlying ELFEFs' effects, we extended our studies to an in vitro model of neural stem cells (NSCs) isolated from the hippocampi of newborn mice. We found that ELFEFs enhanced proliferation and neuronal differentiation of hippocampal NSCs by regulation of epigenetic mechanisms leading to pro-neuronal gene expression. Upon ELFEF stimulation of NSCs, we observed a significant enhancement of expression of the pro-proliferative gene hairy enhancer of split 1 and the neuronal determination genes NeuroD1 and Neurogenin1. These events were preceded by increased acetylation of H3K9 and binding of the phosphorylated transcription factor cAMP response element-binding protein (CREB) on the regulatory sequence of these genes. Such ELFEF-dependent epigenetic modifications were prevented by the Ca_v1-channel blocker nifedipine, and were associated with increased occupancy of CREB-binding protein (CBP) to the same loci within the analyzed promoters. Our results unravel the molecular mechanisms underlying the ELFEFs' ability to improve endogenous neurogenesis, pointing to histone acetylation-related chromatin remodeling as a critical determinant. These findings could pave the way to the development of novel therapeutic approaches in regenerative medicine.

(NE) Li L, Xiong DF, Liu JW, Li ZX, Zeng GC, Li HL. No effects of power line frequency extremely low frequency electromagnetic field exposure on selected neurobehavior tests of workers inspecting transformers and distribution line stations versus controls. Australas Phys Eng Sci Med. 2013 Dec 31. [Epub ahead of print] (HU, BE, CE)

We aimed to evaluate the interference of 50 Hz extremely low frequency electromagnetic field (ELF-EMF) occupational exposure on the neurobehavior tests of workers performing tour-inspection close to transformers and distribution power lines. Occupational short-term "spot" measurements were carried out. 310 inspection workers and 300 logistics staff were selected as exposure and control. The neurobehavior tests were performed through computer-based neurobehavior evaluation system, including mental arithmetic, curve coincide, simple visual reaction time, visual retention, auditory digit span and pursuit aiming. In 500 kV areas electric field intensity at 71.98 % of total measured 590 spots were above 5 kV/m (national occupational standard), while in 220 kV areas electric field intensity at 15.69 % of total 701 spots were above 5 kV/m. Magnetic field flux density at all the spots was below 1,000 μT (ICNIRP occupational standard). The neurobehavior score changes showed no statistical significance. Results of neurobehavior tests among different age, seniority groups showed no significant changes. Neurobehavior changes caused by daily repeated ELF-EMF exposure were not observed in the current study.

(NE) Li Y, Zhang C, Song T. Disturbance of the magnetic field did not affect spatial memory. Physiol Res. 2014 Feb 24. [Epub ahead of print] (AS, CE, BE)

Extremely low-frequency magnetic field (ELF-MF) has been suggested to influence the cognitive capability and has to be dynamically evaluated in a longitudinal study. Previous training can affect performance, but the influence under magnetic field is unclear. This study aims to evaluate the effects of previous training and ELF-MF exposure on learning and memory using the Morris water maze (MWM). Sprague-Dawley rats were subjected to MWM training, ELF-MF exposure (50 Hz, 100 microT), or ELF-MF exposure combined with MWM training for 90 days. Normal rats were used as controls. The MWM was used to test. The data show that the rats exposed to training and ELF-MF with training performed better on spatial acquisition when re-tested. However, during the probe trial the rats showed no change between the training phase and the test phase. Compared with the control group, the ELF-MF group showed no significant differences. These results confirm that previous training can improve the learning and memory capabilities regarding spatial acquisition in the MWM and this effect can last for at least 90 days. However, this improvement in learning and memory capabilities was not observed during the probe trial. Furthermore, ELF-MF exposure did not interfere with the improvement in learning and memory capabilities.

(E) Liu T, Wang S, He L, Ye K. Anxiogenic effect of chronic exposure to extremely low frequency magnetic field in adult rats. Neurosci Lett. 434(1):12-17, 2008a. (AS, CE, BE)

Previous study has suggested some relations between extremely low frequency magnetic field (ELF MF) and the emotional state of human beings and animals. The aim of the present study was to investigate whether the anxiety level could be affected by repeated ELF MF exposure of different daily durations. Adult SD rats were submitted to no exposure, MF exposure 1h/day or 4h/day for 25 days. Anxiety-related behaviors were examined in the open field test (OFT), the elevated plus maze (EPM), and light/dark box on the 21th, 23th and 25th exposure day, respectively. Results demonstrated that MF exposure 4h/day increased the anxiety-like behaviors in rats in the open field test and the elevated plus maze test, without altering their locomotor activity, but had no effect in the light/dark box test. Moreover, MF exposure 1h/day had no effect in any test. These findings indicate that chronic ELF MF exposure has anxiogenic effect in rats, which is dependent on the daily exposure duration and it is more sensitive to void space than to strong light.

(E) Liu T, Wang S, He L, Ye K. Chronic exposure to low-intensity magnetic field improves acquisition and maintenance of memory. Neuroreport. 19(5):549-552, 2008b. (AS, CE, BE)

Although past research has suggested that acute exposure to extremely low-frequency magnetic field (ELF MF) impairs learning and memory function, data on chronic exposure remain scarce. In this study, we examined the changes in spatial learning and memory by the Morris water maze test after 4 weeks of daily exposure of rats to a 50-Hz magnetic field of 2 mT for either 1 or 4 h. We found that chronic exposure to ELF MF reduced the latency to find the hidden platform and improved long-term memory of former location of platform without affecting the short-term memory and motor activity. These findings for the first time indicate that chronic exposure to ELF MF exerts a positive effect on the acquisition and maintenance of spatial memory.

(E) Maestú C, Blanco M, Nevado A, Romero J, Rodríguez-Rubio P, Galindo J, Bautista Lorite J, de las Morenas F, Fernández-Argüelles P. Reduction of pain thresholds in fibromyalgia after very low-intensity magnetic stimulation: a double-blinded, randomized placebo-controlled clinical trial. Pain Res Manag. 18(6):e101-106, 2013. (HU, BE, MA)

BACKGROUND: Exposure to electromagnetic fields has been reported to have analgesic and antinociceptive effects in several organisms. Objective: To test the effect of very low-intensity transcranial magnetic stimulation on symptoms associated with fibromyalgia syndrome. METHODS: A double-blinded, placebo-controlled clinical trial was performed in the Sagrado Corazón Hospital, Seville, Spain. Female fibromyalgia patients (22 to 50 years of age) were randomly assigned to either a stimulation group or a sham group. The stimulation group (n=28) was stimulated using 8 Hz pulsed magnetic fields of very low intensity, while the sham group (n=26) underwent the same protocol without stimulation. Pressure pain thresholds before and after stimulation were determined using an algometer during the eight consecutive weekly sessions of the trial. In addition, blood serotonin levels were measured and patients completed questionnaires to monitor symptom evolution. RESULTS: A repeated-measures ANOVA indicated statistically significant improvement in the stimulation group compared with the control group with respect to somatosensory pain thresholds, ability to perform daily activities, perceived chronic pain and sleep quality. While improvement in pain thresholds was apparent after the first stimulation session, improvement in the other three measures occurred after the sixth week. No significant between-group differences were observed in scores of depression, fatigue, severity of headaches or serotonin levels. No adverse side effects were reported in any of the patients. CONCLUSIONS: Very low-intensity magnetic stimulation may represent a safe and effective treatment for chronic pain and other symptoms associated with fibromyalgia.

(E) Manikonda PK, Rajendra P, Devendranath D, Gunasekaran B, Channakeshava, Aradhya RS, Sashidhar RB, Subramanyam C. Influence of extremely low frequency magnetic fields on Ca2+ signaling and NMDA receptor functions in rat hippocampus. Neurosci Lett. 413(2):145-149, 2007. (AS, CE, CC)

Extremely low frequency (ELF<300Hz) electromagnetic fields affect several neuronal activities including memory. Because ELF magnetic fields cause altered Ca(2+) homeostasis in neural tissues, we examined their influence on Ca(2+) signaling enzymes in hippocampus and related them with NMDA receptor functions. Hippocampal regions were obtained from brains of 21-day-old rats that were exposed for 90 days to 50Hz magnetic fields at 50 and 100 microT intensities. In comparison to controls, ELF exposure caused increased intracellular Ca(2+) levels concomitant with increased activities of Ca(2+)-dependent protein kinase C (PKC), cAMP-dependent protein kinase and calcineurin as well as decreased activity of Ca(2+)-calmodulin-dependent protein kinase in hippocampal regions. Simultaneous ligand-binding studies revealed decreased binding to N-methyl-D-aspartic acid (NMDA) receptors. The combined results suggest that perturbed neuronal functions caused by ELF exposure may involve altered Ca(2+) signaling events contributing to aberrant NMDA receptor activities.

(E) Manikonda PK, Rajendra P, Devendranath D, Gunasekaran B, Channakeshava, Aradhya SR, Sashidhar RB, Subramanyam C. Extremely low frequency magnetic fields induce oxidative stress in rat brain. Gen Physiol Biophys. 2013 Dec 13. [Epub ahead of print] (AS, CE, OX, CC)

The present investigation was conducted to understand the influence of long-term exposure of rats to extremely low frequency magnetic fields (ELF-MF), focusing on oxidative stress (OS) on different regions of rat's brain. Male Wistar rats (21-day-old) were exposed to ELF-MF (50 Hz;

50 and 100 μT) for 90 days continuously; hippocampal, cerebellar and cortical regions from rats were analyzed for (i) reactive oxygen species (ROS), (ii) metabolites indicative of OS and (iii) antioxidant enzymes. In comparison to control group rats, the rats that were continuously exposed to ELF-MF caused OS and altered glutathione (GSH/GSSG) levels in dose-dependent manner in all the regions of the brain. Accumulation of ROS, lipid peroxidation end products and activity of superoxide dismutase in different regions was in the descending order of cerebellum < hippocampus < cortex. Decrement in GSH/GSSG levels and increment in glutathione peroxidase activity were in the descending order of hippocampus < cerebellum < cortex. The continuous exposure to ELF-MF caused OS in all the examined regions of brain more significantly at 100 μT than at 50 μT. Varied influences observed in different regions of the brain, as documented in this study, may contribute to altered metabolic patterns in its related regions of the central nervous system, leading to aberrant neuronal functions.

(E) Manjhi J, Kumar S, Behari J, Mathur R. Effect of extremely low frequency magnetic field in prevention of spinal cord injury-induced osteoporosis. J Rehabil Res Dev. 50(1):17-30, 2013. (AS, CE, MA)

The present study was designed to investigate the effect of extremely low frequency (ELF) magnetic field (MF) on spinal cord injury (SCI)-induced osteoporosis in rats. Adult male Wistar rats (n = 24) were equally divided into sham, SCI, and SCI+MF groups. Complete transection of spinal cord (thoracic 11 vertebra) was surgically performed under anesthesia, whereas in the sham group only laminectomy was done. Post-SCI day 1, rats were either exposed (2 h/d × 8 wk) to ELF-MF (17.96 micro-Tesla, 50 Hz; SCI+MF group) or sham exposed (SCI group). Basso, Beattie, and Bresnahan (BBB) score was recorded weekly. All the rats were sacrificed 8 wk post-SCI; tibia and femur bones were isolated for the analysis of bone mineral content (BMC; total calcium [Ca], phosphorus [P], carbon [C]), bone mineral density (BMD), and biochemical status (osteocalcin, collagen I, alkaline phosphatase). The BBB score decreased post-SCI, which partially recovered after ELF-MF. In SCI rats, there was a statistically significant decrease in BMC, Ca, P, C, BMD, and biochemical level in both the bones as compared with the sham group, which was attenuated in SCI+MF rats except the C content. Electron microscopic study revealed the enhancement of microstructural composition and compactness in cortical and trabecular parts of treated bones. The results suggest that the chronic (2 h/d × 8 wk) ELF-MF exposure (17.96 micro-Tesla, 50 Hz) to SCI rats is effective in attenuating SCI-induced osteoporosis.

(E) Martínez-Sámano J, Torres-Durán PV, Juárez-Oropeza MA, Verdugo-Díaz L. Effect of acute extremely low frequency electromagnetic field exposure on the antioxidant status and lipid levels in rat brain. Arch Med Res. 43(3):183-189, 2012. (AS, AE, CC, OX)

BACKGROUND AND AIMS: It is generally accepted that electromagnetic fields (EMF) can exert biological effects; however, the mechanisms by which EMF elicits responses are still unknown. The present study was designed to assess the immediate effects of acute EMF exposure, movement restriction, and the combination of both on the antioxidant systems and lipid content in the whole brain of rat. METHODS: Thirty two male Wistar rats were arranged in four groups: control, EMF exposed, movement restrained (MR), and EMF + MR for 2 h. Rats were then sacrificed and their brains analyzed for superoxide dismutase and catalase activities, reduced glutathione, nitric oxide, total cholesterol, and triacylglycerol levels, as well as plasma corticosterone concentrations. RESULTS: Acute exposure to EMF induces reduction in catalase and superoxide dismutase activities, whereas the combination of EMF + MR also decreases both reduced glutathione and nitric oxide levels. Our results show that the acute exposure to EMF does not induce elevation of stress-hormone corticosterone but impairs the antioxidant status in rat brain. CONCLUSIONS: Plasma corticosterone concentration and antioxidant data indicate that the acute exposure to EMF appears to be a mild stressor that leads to some adaptive responses due to the activation of systems controlling the brain oxidative balance.

(NE) Masuda H, de Gannes FP, Haro E, Billaudel B, Ruffié G, Lagroye I, Veyret B. Lack of effect of 50-Hz magnetic field exposure on the binding affinity of serotonin for the 5-HT 1B receptor subtype. Brain Res. 1368:44-51, 2011. (CS, AE, CC)

There is some concern that exposure to extremely low-frequency magnetic fields (MF) causes adverse health effects via signal transduction pathways. Two previous studies reported that exposure to 50-Hz MF decreased the binding affinity of the 1B receptor subtype of serotonin (5-HT) in rat brain membranes. The aim of this study was to investigate whether the exposure to MF affects binding to the 5-HT(1B) receptor and a physiological function associated with 5-HT(1B) receptor activation. Rat brain crude membrane fractions, including 5-HT(1B) receptor and C6-glial cells transfected with human 5-HT(1B) receptor gene, were exposed to 50-Hz MF at 1 mT using Merritt coils under temperature-regulated conditions. In the rat crude membrane, there was no significant difference in the affinity constant of [(3)H]-5-HT between exposed (K(d): 0.92±0.38 nM) and sham-exposed (K(d): 1.00±0.32 nM). The lack of affinity change after exposure was also confirmed using a chemical agonist of the 5-HT receptor, [(3)H]-5-carboxytryptamine (K(d): 0.59±0.06 nM for exposed and 0.71±0.08 nM for sham). Similar negative results in terms of affinity constant were obtained on the human 5-HT(1B) receptor in C6-glial cells. In addition, forskolin-stimulated cAMP production was inhibited by 5-HT administration in a dose-dependent manner in C6-glial cells, but exposure did not modify the inhibitory response. This study thus failed to confirm the previous results and findings suggest that exposure to MF below the current occupational limit does not affect the physiological function involved in 5-HT(1B) receptor subtypes.

(E) Partsvania B, Sulaberidze T, Modebadze Z, Shoshiashvili L. Extremely low-frequency magnetic fields effects on the snail single neurons. Electromagn Biol Med. 27(4):409-417, 2008. (CS, EE)

The aim of present work is to explore the influence of extremely low-frequency electromagnetic fields (8.34 and 217 Hz) utilized in cell phones on habituation of the mollusk single neuron to intracellular stimuli. The isolated nervous system of the mollusk Helix Pomatia was used in the experiments. Helmholtz coils were used to expose brain ganglia to the low-frequency electromagnetic fields. Peak values of the extremely low-frequency fields were between 1 and 6 mT. Neuron electrophysiology was investigated using a standard microelectrode technique. Exposure of the neuron to the low-frequency electromagnetic fields caused dehabituation to intracellular stimulus. The effect was proportional to the magnetic induction peak value. The observed dehabituation occurs by degradation of the signal to noise ratio and by alteration of the neuron's normal function.

(E) Perentos N, Croft RJ, McKenzie RJ, Cvetkovic D, Cosic I. The effect of GSM-like ELF radiation on the alpha band of the human resting EEG. Conf Proc IEEE Eng Med Biol Soc. 2008:5680-5683, 2008. (HU, EE)

Mobile phone handsets such as those operating in the GSM network emit extremely low frequency electromagnetic fields ranging from DC to at least 40 kHz. As a subpart of an extended protocol, the influence of these fields on the human resting EEG has been investigated in a fully counter balanced, double blind, cross-over design study that recruited 72 healthy volunteers. A decrease in the alpha frequency band was observed during the 20 minutes of ELF exposure in the exposed hemisphere only. This result suggests that ELF fields as emitted from GSM handsets during the DTX mode may have an effect on the resting alpha band of the human EEG.

(E) Piacentini R, Ripoli C, Mezzogori D, Azzena GB, Grassi C. Extremely low-frequency electromagnetic fields promote in vitro neurogenesis via upregulation of Ca(v)1-channel activity. J Cell Physiol. 215(1):129-139, 2008. (CS, AE, MC, MA)

We previously reported that exposure to extremely low-frequency electromagnetic fields (ELFEFs) increases the expression and function of voltage-gated Ca2+)channels and that Ca2+ influx through Ca(v)1 channels plays a key role in promoting the neuronal differentiation of neural stem/progenitor cells (NSCs). The present study was conducted to determine whether ELFEFs influence the neuronal differentiation of NSCs isolated from the brain cortices of newborn mice by modulating Ca(v)1-channel function. In cultures of differentiating NSCs exposed to ELFEFs (1 mT, 50 Hz), the percentage of cells displaying immunoreactivity for neuronal markers (beta-III-tubulin, MAP2) and for Ca(v)1.2 and Ca(v)1.3 channels was markedly increased. NSC-differentiated neurons in ELFEF-exposed cultures also exhibited significant increases in spontaneous firing, in the percentage of cells exhibiting Ca2+ transients in response to KCl stimulation, in the amplitude of these transients and of Ca2+ currents generated by the activation of Ca(v)1 channels. When the Ca(v)1-channel blocker nifedipine (5 microM) was added to the culture medium, the neuronal yield of NSC differentiation dropped significantly, and ELFEF exposure no longer produced significant increases in beta-III-tubulin- and MAP2-immunoreactivity rates. In contrast, the effects of ELFEFs were preserved when NSCs were cultured in the presence of either glutamate receptor antagonists or Ca(v)2.1- and Ca(v)2.2-channel blockers. ELFEF stimulation during the first 24 h of differentiation caused Ca(v)1-dependent increases in the number of cells displaying CREB phosphorylation. Our data suggest that ELFEF exposure promotes neuronal differentiation of NSCs by upregulating Ca(v)1-channel expression and function.

(E) Podda MV, Leone L, Barbati SA, Mastrodonato A, Li Puma DD, Piacentini R, Grassi C. Extremely low-frequency electromagnetic fields enhance the survival of newborn neurons in the mouse hippocampus. Eur J Neurosci. 2013 Dec 30. doi: 10.1111/ejn.12465. [Epub ahead of print] (AS, CS, CE, AE, BE, CC, MA)

In recent years, much effort has been devoted to identifying stimuli capable of enhancing adult neurogenesis, a process that generates new neurons throughout life, and that appears to be dysfunctional in the senescent brain and in several neuropsychiatric and neurodegenerative diseases. We previously reported that in vivo exposure to extremely low-frequency electromagnetic fields (ELFEFs) promotes the proliferation and neuronal differentiation of hippocampal neural stem cells (NSCs) that functionally integrate in the dentate gyrus. Here, we extended our studies to specifically assess the influence of ELFEFs on hippocampal newborn cell survival, which is a very critical issue in adult neurogenesis regulation. Mice were injected with 5-bromo-2'-deoxyuridine (BrdU) to label newborn cells, and were exposed to ELFEFs 9 days later, when the most dramatic decrease in the number of newly generated neurons occurs. The results showed that ELFEF exposure (3.5 h/day for 6 days) enhanced newborn neuron survival as documented by double staining for BrdU and doublecortin, to identify immature neurons, or NeuN labeling of mature neurons. The effects of ELFEFs were associated with enhanced spatial learning and memory. In an in vitro model of hippocampal NSCs, ELFEFs exerted their pro-survival action by rescuing differentiating neurons from apoptotic cell death. Western immunoblot assay revealed reduced expression of the pro-apoptotic protein Bax, and increased levels of the anti-apoptotic protein Bcl-2, in the hippocampi of ELFEF-exposed mice as well as in ELFEF-exposed NSC cultures, as compared with their sham-exposed counterparts. Our results may have clinical implications for the treatment of impaired neurogenesis associated with brain aging and neurodegenerative diseases.

(E) Rauš S, Selaković V, Manojlović-Stojanoski M, Radenović L, Prolić Z, Janać B. Response of Hippocampal Neurons and Glial Cells to Alternating Magnetic Field in Gerbils Submitted to Global Cerebral Ischemia. Neurotox Res. 23(1):79-91, 2013. (AS, CE, MC, MA)

The purpose of this study was to determine whether exposure to an extremely low-frequency magnetic field (ELF-MF, 50 Hz) affects the outcome of postischemic damage in the hippocampus of Mongolian gerbils. After 10-min bilateral carotid occlusion, the gerbils were continuously exposed to ELF-MF (average magnetic induction at the center of the cage was 0.5 mT) for 7 days. The impact of ELF-MF was estimated immediately (the 7th day after reperfusion) and 7 days after cessation of exposure (the 14th day after reperfusion) compared with ischemic gerbils without ELF-MF exposure. Applying stereological methods, histological evaluation of changes in the hippocampus was done for determining its volume, volume densities of degenerating neurons and astrocytes, as well as the number of microglial cells per unit area. ELF-MF per se did not induce any morphological changes, while 10-min global cerebral ischemia led to neuronal death, especially in CA1 region of the hippocampus, as expected. Ischemic gerbils exposed to ELF-MF had significantly a lower degree of cell loss in the examined structure and greater responses of astrocytes and microglial cells than postischemic gerbils without exposure on the seventh day after reperfusion (immediate effect of ELF-MF). Similar response was observed on the 14th day after reperfusion (delayed effect of ELF-MF); however, differences in measured parameters were low and insignificant. Applied ELF-MF has possible neuroprotective function in the hippocampus, as the most sensitive brain structure in the model of global cerebral ischemia, through reduction of neuronal death and activation of astrocytes and microglial cells.

(E) Rauš S, Selaković V, Radenović L, Prolić Z, Janać B. Extremely low frequency magnetic field induced changes in motor behaviour of gerbils submitted to global cerebral ischemia. Behav Brain Res. 228(2):241-246, 2012. (AS, CE, BE, MA)

The purpose of this study was to evaluate behavioural effects of an extremely low frequency magnetic field (ELF-MF) in 3-month-old Mongolian gerbils submitted to global cerebral ischemia. After 10-min occlusion of both common carotid arteries, the gerbils were placed in the vicinity of an electromagnet and continuously exposed to ELF-MF (50Hz, 0.5mT) for 7 days. Their behaviour (locomotion, stereotypy, rotations, and immobility) was monitored on days 1, 2, 4, 7, and 14 after reperfusion for 60 min in the open field. It was shown that the 10-min global cerebral ischemia per se induced a significant motor activity increase (locomotion, stereotypy and rotations), and consequently immobility decrease until day 4 after reperfusion, compared to control gerbils. Exposure to ELF-MF inhibited development of ischemia-induced motor hyperactivity during the whole period of registration, but significantly in the first 2 days after reperfusion, when the postischemic hyperactivity was most evident. Motor activity of these gerbils was still significantly increased compared to control ones, but only on day 1 after reperfusion. Our results revealed that the applied ELF-MF (50Hz, 0.5mT) decreased motor hyperactivity induced by the 10-min global cerebral ischemia, via modulation of the processes that underlie this behavioural response.

(E) Rauš Balind S, Selaković V, Radenović L, Prolić Z, Janać B.Extremely Low Frequency Magnetic Field (50 Hz, 0.5 mT) Reduces Oxidative Stress in the Brain of Gerbils Submitted to Global Cerebral Ischemia. PLoS One. 2014 Feb 19;9(2):e88921. doi: 10.1371/journal.pone.0088921. eCollection 2014. (AS, CE, OX, CC, MA)

Magnetic field as ecological factor has influence on all living beings. The aim of this study was to determine if extremely low frequency magnetic field (ELF-MF, 50 Hz, 0.5 mT) affects oxidative stress in the brain of gerbils submitted to 10-min global cerebral ischemia. After occlusion of both carotid arteries, 3-month-old gerbils were continuously exposed to ELF-MF for 7 days. Nitric oxide and superoxide anion production, superoxide dismutase activity and index of lipid peroxidation were examined in the forebrain cortex, striatum and hippocampus on the 7(th) (immediate effect of ELF-MF) and 14(th) day after reperfusion (delayed effect of ELF-MF). Ischemia per se increased oxidative stress in the brain on the 7(th) and 14(th) day after reperfusion. ELF-MF also increased oxidative stress, but to a greater extent than ischemia, only immediately after cessation of exposure. Ischemic gerbils exposed to ELF-MF had increased oxidative stress parameters on the 7(th) day after reperfusion, but to a lesser extent than ischemic or ELF-MF-exposed animals. On the 14(th) day after reperfusion, oxidative stress parameters in the brain of these gerbils were mostly at the control levels. Applied ELF-MF decreases oxidative stress induced by global cerebral ischemia and thereby reduces possible negative consequences which free radical species could have in the brain. The results presented here indicate a beneficial effect of ELF-MF (50 Hz, 0.5 mT) in the model of global cerebral ischemia.

(E) Ravera S, Bianco B, Cugnoli C, Panfoli I, Calzia D, Morelli A, Pepe IM. Sinusoidal ELF magnetic fields affect acetylcholinesterase activity in cerebellum synaptosomal membranes. Bioelectromagnetics. 31(4):270-276, 2010. (CS, AE, CE)

The effects of extremely low frequency magnetic fields (ELF-MF) on acetylcholinesterase (AChE) activity of synaptosomal membranes were investigated. Sinusoidal fields with 50 Hz frequency and different amplitudes caused AChE activity to decrease about 27% with a threshold of about 0.74 mT. The decrease in enzymatic activity was independent of the time of permanence in the field and was completely reversible. Identical results were obtained with exposure to static MF of the same amplitudes. Moreover, the inhibitory effects on enzymatic activity are spread over frequency windows with different maximal values at 60, 200, 350, and 475 Hz. When synaptosomal membranes were solubilized with Triton, ELF-MF did not affect AChE activity, suggesting the crucial role of the membrane, as well as the lipid linkage of the enzyme, in determining the conditions for inactivation. The results are discussed in order to give an interpretation at molecular level of the macroscopic effects produced by ELF-MF on biological systems, in particular the alterations of embryo development in many organisms due to acetylcholine accumulation.

****(E) Rageh MM, El-Gebaly RH, El-Bialy NS. Assessment of genotoxic and cytotoxic hazards in brain and bone marrow cells of newborn rats exposed to extremely low-frequency magnetic field. J Biomed Biotechnol. 2012;2012:716023. (AS, CE, OX, DE)**

The present study aimed to evaluate the association between whole body exposure to extremely low frequency magnetic field (ELF-MF) and genotoxic , cytotoxic hazards in brain and bone marrow cells of newborn rats. Newborn rats (10 days after delivery) were exposed continuously to 50 Hz, 0.5 mT for 30 days. The control group was treated as the exposed one with the sole difference that the rats were not exposed to magnetic field. Comet assay was used to quantify the level of DNA damage in isolated brain cells. Also bone marrow cells were flushed out to assess micronucleus induction and mitotic index. Spectrophotometric methods were used to measure the level of malondialdehyde (MDA) and the activity of glutathione (GSH) and superoxide dismutase (SOD). The results showed a significant increase in the mean tail moment indicating DNA damage in exposed group ($P < 0.01, 0.001, 0.0001$). Moreover ELF-MF exposure induced a significant ($P < 0.01, 0.001$) four folds increase in the induction of micronucleus and about three folds increase in mitotic index ($P < 0.0001$). Additionally newborn rats exposed to ELF-MF showed significant higher levels of MDA and SOD ($P < 0.05$). Meanwhile ELF-MF failed to alter the activity of GSH. In conclusion, the present study suggests an association between DNA damage and ELF-MF exposure in newborn rats.

(E) Reyes-Guerrero G, Guzmán C, García DE, Camacho-Arroyo I, Vázquez-García M. Extremely low-frequency electromagnetic fields differentially regulate estrogen receptor-alpha and -beta expression in the rat olfactory bulb. Neurosci Lett. 471(2):109-13, 2010. (AS, AE, CC)

Recently, the effects of extremely low-frequency electromagnetic fields (ELF EMF) on biological systems have been extensively investigated. In this report, the influence of ELF EMF on olfactory bulb (OB) estrogen receptor-alpha (ER alpha) mRNA and -beta (ER beta) mRNA expression was studied by RT-PCR in adult female and male rats. Results reveal for the first time that ELF EMF exerted a biphasic effect on female OB ER beta mRNA gene expression, which increased during diestrous and decreased during estrous. We did not observe any influence of ELF EMF on female OB ER alpha mRNA expression. Our data demonstrate a fluctuating pattern of ER-alpha and -beta mRNA expression in the female OB throughout the phases of the estrous cycle in non-ELF EMF-exposed animals. Thus the highest ER alpha expression was observed in diestrous and the lowest in proestrous. The pattern of ER beta mRNA was less variable, the lowest expression was observed in diestrous. ER-alpha mRNA and -beta mRNA expression level in the male OB did not exhibit any variation either in ELF EMF-exposed or non-ELF EMF-exposed animals. In summary, ELF EMF modulate ER beta gene expression in the OB of female adult rats but not in males.

(E) Ross ML, Koren SA, Persinger MA. Physiologically patterned weak magnetic fields applied over left frontal lobe increase acceptance of false statements as true. Electromagn Biol Med. 27(4):365-371, 2008. (HU, AE, BE)

Fifty men and women were exposed to only one of four experimentally generated magnetic fields over the left prefrontal region (above the eyebrow) or to a sham field immediately after the words "true" or "false" were presented following statements of definitions of words for a "foreign language". Three of the patterns (25 Hz, 50 Hz, or burst-firing) with intensities between 1 and 10 microT were presented for 1 s during the refutation process (immediately after the offset of "true" or "false") for specific statements from a total of 28 statements. The fourth pattern was a variable approximately 7-10 Hz (10 nT) field generated from the circuitry that was present continuously during the entire experiment. When the statements were presented again, the groups who had received the burst-firing ("limbic") or 25 Hz pulsed magnetic fields during the refutation process accepted about twice the number of false statements as true compared to those exposed to the 50 Hz field or sham-field conditions. The treatments did not significantly affect the numbers of true statements accepted as false. These results suggest that the appropriately pulsed magnetic field during the refutation process of what one has been told or has heard can increase the probability a person will accept a false statement as true.

(E) Salunke BP, Umathe SN, Chavan JG. Involvement of NMDA receptor in low-frequency magnetic field-induced anxiety in mice. Electromagn Biol Med. 2013 Oct 16. [Epub ahead of print] (AS, CE, CC, BE)

It had been reported that exposure to extremely low-frequency magnetic field (ELFMF) induces anxiety in human and rodents. Anxiety mediates via the activation of N-methyl-d-aspartate (NMDA) receptor, whereas activation of γ-aminobutyric acid (GABA) receptor attenuates the same. Hence, the present study was carried out to understand the contribution of NMDA and/or GABA receptors modulation in ELFMF-induced anxiety for which Swiss albino mice were exposed to ELFMF (50 Hz, 10 G) by subjecting them to Helmholtz coils. The exposure was for 8 h/day for 7, 30, 60, 90 and 120 days. Anxiety level was assessed in elevated plus maze, open field test and social interaction test, on 7th, 30th, 60th, 90th and 120th exposure day, respectively. Moreover, the role of GABA and glutamate in ELFMF-induced anxiety was assessed by treating mice with muscimol [0.25 mg/kg intraperitoneally (i.p.)], bicuculline (1.0 mg/kg i.p.), NMDA (15 mg/kg i.p.) and MK-801 (0.03 mg/kg i.p.), as a $GABA_A$ and NMDA receptor agonist and antagonist, respectively. Glutamate receptor agonist exacerbated while inhibitor attenuated the ELFMF-induced anxiety. In addition, levels of GABA and glutamate were determined in regions of the brain viz, cortex, striatum, hippocampus and hypothalamus. Experiments demonstrated significant elevation of GABA and glutamate levels in the hippocampus and hypothalamus. However, GABA receptor modulators did not produce significant effect on ELFMF-induced anxiety and elevated levels of GABA at tested dose. Together, these findings suggest that ELFMF significantly induced anxiety behavior, and indicated the involvement of NMDA receptor in its effect.

(E) Schmid MR, Murbach M, Lustenberger C, Maire M, Kuster N, Achermann P, Loughran SP. Sleep EEG alterations: effects of pulsed magnetic fields versus pulse-modulated radio frequency electromagnetic fields. J Sleep Res. 2012 Jun 22. doi: 10.1111/j.1365-2869.2012.01025.x. [Epub ahead of print] (HU, AE, EE)

Studies have repeatedly shown that electroencephalographic power during sleep is enhanced in the spindle frequency range following radio frequency electromagnetic field exposures pulse-modulated with fundamental frequency components of 2, 8, 14 or 217 Hz and combinations of these. However, signals used in previous studies also had significant harmonic components above 20 Hz. The current study aimed: (i) to determine if modulation components above 20 Hz, in combination with radio frequency, are necessary to alter the electroencephalogram; and (ii) to test the demodulation hypothesis, if the same effects occur after magnetic field exposure with the same pulse sequence used in the pulse-modulated radio frequency exposure. In a randomized double-blind crossover design, 25 young healthy men were exposed at weekly intervals to three different conditions for 30 min before sleep. Cognitive tasks were also performed during exposure. The conditions were a 2-Hz pulse-modulated radio frequency field, a 2-Hz pulsed magnetic field, and sham. Radio frequency exposure increased electroencephalogram power in the spindle frequency range. Furthermore, delta and theta activity (non-rapid eye movement sleep), and alpha and delta activity (rapid eye movement sleep) were affected following both exposure conditions. No effect on sleep architecture and no clear impact of exposure on cognition was observed. These results demonstrate that both pulse-modulated radio frequency and pulsed magnetic fields affect brain physiology, and the presence of significant frequency components above 20 Hz are not fundamental for these effects to occur. Because responses were not identical for all exposures, the study does not support the hypothesis that effects of radio frequency exposure are based on demodulation of the signal only.

(E) Selaković V, Rauš Balind S, Radenović L, Prolić Z, Janać B. Age-Dependent Effects of ELF-MF on Oxidative Stress in the Brain of Mongolian Gerbils. Cell Biochem Biophys. 66(3):513-521, 2013. (AS, CE, OX)

The aim of study was to investigate the effects of extremely low frequency magnetic field (ELF-MF; 50 Hz; 0.1, 0.25 and 0.5 mT) on oxidative stress in the brain of 3- (adult) and 10-month-old (middle-aged) gerbils. Nitric oxide (NO) level, superoxide (O(2) (-)) production, superoxide dismutase (SOD) activity, and index of lipid peroxidation (ILP) were measured in the forebrain cortex, striatum, hippocampus, and cerebellum immediately and 3 days after cessation of 7-day exposure. In all gerbils, ELF-MF significantly increased oxidative stress in all tested brain regions. This effect was correlated with the value of magnetic induction and was higher in middle-aged gerbils. Three days after cessation of exposure, the values of examined parameters were closer to control levels. In adult gerbils, the effect of ELF-MF of 0.1 mT on NO level, O(2) (-) production and SOD activity was almost fully disappeared, and ILP was at the control level regardless of the value of magnetic induction. In middle-aged gerbils, the effect of ELF-MF was still present but to a lesser degree than those observed immediately after cessation of exposure. These findings pointed out the ability of ELF-MF to induce age- and magnetic induction-dependent modification of oxidative stress in the brain.

(E) Shafiei SA, Firoozabadi SM, Rasoulzadeh Tabatabaie K, Ghabaee M. Study of the frequency parameters of EEG influenced by zone-dependent local ELF-MF exposure on the human head. Electromagn Biol Med. 31(2):112-12, 2012. (HU, AE, EE)

It has been reported that human subjects exposed to electromagnetic fields exhibit changes in human EEG signals at the frequency of stimulation. The aim of the present study was to expose different parts of the brain to extremely low-frequency magnetic fields locally and investigate EEG power spectrum alters at the frequency of stimulation. EEG relative power spectrum were evaluated at 3, 5, 10, 17, and 45 Hz frequencies at T4, T3, F3, Cz, and F4 points, respectively, when these points were exposed to magnetic fields with similar frequencies and 100 μT intensity. The paired t-test results showed that power value of EEG did not alter significantly at the frequency of stimulation ($P<0.05$). Further, significant changes in different EEG bands caused by locally exposing to ELF-MF in different points of brain were observed. The changes in the EEG bands were not limited necessarily to the exposure point.

(E) Shin EJ, Jeong JH, Kim HJ, Jang CG, Yamada K, Nabeshima T, Kim HC. Exposure to extremely low frequency magnetic fields enhances locomotor activity via activation of dopamine D1-like receptors in mice. J Pharmacol Sci. 105(4):367-371, 2007. (AS, AE, CE, BE, CC)

We demonstrated that exposure to extremely low frequency magnetic fields (ELF-MF) enhanced dopamine levels in the rat striatum. To extend our understanding, we examined the role of dopaminergic receptors in ELF-MF-induced behavioral changes. Exposure to ELF-MF (2.4 mT, 1 h/day, for one or seven days) enhanced locomotor activity in a time-dependent manner. This hyperlocomotor activity paralleled an increase in c-Fos-like immunoreactivity (c-Fos-IR). Pretreatment with SCH23390, a dopaminergic D(1)-like receptor antagonist, but not with sulpiride, a dopaminergic D(2)-like receptor antagonist, inhibited ELF-MF-induced increased locomotor activity and c-Fos-IR. Thus, our results suggest that ELF-MF-induced behavioral responses are, at least in part, mediated by activation of dopamine D(1)-like receptors.

(E) Shin EJ, Nguyen XK, Nguyen TT, Pham DT, Kim HC. Exposure to extremely low frequency magnetic fields induces fos-related antigen-immunoreactivity via activation of dopaminergic D1 receptor. Exp Neurobiol. 20(3):130-6, 2011. (CE, BE, CC)

We previously demonstrated that repeated exposure to extremely low frequency magnetic fields (ELF-MF) increases locomotor activity via stimulation of dopaminergic D1 receptor (J. Pharmacol. Sci., 2007;105:367-371). Since it has been demonstrated that activator protein-1 (AP-1) transcription factors, especially 35-kDa fos-related antigen (FRA), play a key role in the neuronal and behavioral adaptation in response to various stimuli, we examined whether repeated ELF-MF exposure induces FRA-immunoreactivity (FRA-IR) in the striatum and nucleus accumbens (striatal complex) of the mice. Repeated exposure to ELF-MF (0.3 or 2.4 mT, 1 h/day, for consecutive fourteen days) significantly induced hyperlocomotor activity and FRA-IR in the striatal complex in a field intensity-dependent manner. ELF-MF-induced FRA-IR lasted for at least 1 year, while locomotor activity returned near control level 3 months after the final exposure to ELF-MF. Pretreatment with SCH23390, a dopaminergic D1 receptor antagonist, but not with sulpiride, a dopaminergic D2 receptor antagonist, significantly attenuated hyperlocomotor activity and FRA-IR induced by ELF-MF. Our results suggest that repeated exposure to ELF-MF leads to prolonged locomotor stimulation and long-term expression of FRA in the striatal complex of the mice via stimulation of dopaminergic D1 receptor.

(E) Stevens P. Affective response to 5 microT ELF magnetic field-induced physiological changes. Bioelectromagnetics. 28(2):109-114, 2007. (HU, AE, BE)

Research into effects of weak magnetic fields (MFs) at biologically relevant frequencies has produced ambiguous results. Although they do affect human physiology and behaviour, the direction of effects is inconsistent, with a range of complex and unrelated behaviours being susceptible. A possible explanation is that these effects, rather than being directly caused, are instead related to changes in affective state. A previous study showed that MFs altered the affective content of concurrent perceptions, but it was unclear whether the emotional response was direct or indirect. Here it is shown that exposure to a 0-5 microT MF (DC-offset sinudsoidal wave form) within EEG alpha-band frequencies (8-12 Hz), results in a reported change in emotional state. This relates to a decrease global field power but lacks the frontal alpha-asymmetry that would physiologically indicate a directly induced emotional state, suggesting that participant experiences are due to an interpretation of the effects of MF exposure.

(E) Strasák L, Bártová E, Krejci J, Fojt L, Vetterl V. Effects of ELF-EMF on brain proteins in mice. Electromagn Biol Med. 28(1):96-104, 2009. (AS, AE, CC)

Effect of electromagnetic low frequency fields was studied on mice. We analyzed level of protein in brain of mouse. The levels of c-Jun and c-Fos in brains were measured using Western-blot techniques. Female and male laboratory mice were exposed for 4 days to magnetic field (Bm = 2 mT, f = 50 Hz). The exposure took place in cylindrical coil at laboratory temperature. After the experiment they were sacrificed and the level of protein c-Jun and c-Fos in different parts of brain were estimated. The expression of c-Fos was not affected by magnetic field on the other hand the expression of c-Jun decreased after magnetic field exposure. The results did not depend on sex of mice.

(E) Sun H, Che Y, Liu X, Zhou D, Miao Y, Ma Y. Effects of prenatal exposure to a 50-Hz magnetic field on one-trial passive avoidance learning in 1-day-old chicks. Bioelectromagnetics. 31(2):150-155, 2010. (AS, CE, BE, DE)

We investigated memory impairment in newly hatched chicks following in ovo exposure to a 50-Hz magnetic field (MF) of 2 mT (60 min/day) on embryonic days 12-18. Isolated and paired chicks were used to test the effect of stress during training, and memory retention was tested at 10, 30, and 120 min, following exposure to a bitter-tasting bead (100% methylanthranilate). Results showed that memory was intact at 10 min in both isolated and paired chicks with or without MF exposure. However, while isolated chicks had good memory retention levels at 30 and 120 min, those exposed to MF did not. The results suggest a potential disruption of memory formation following in ovo exposure to MF, with this effect only evident in the more stressed, isolated chicks.

(E) Szemerszky R, Zelena D, Barna I, Bárdos G. Stress-related endocrinological and psychopathological effects of short- and long-term 50Hz electromagnetic field exposure in rats. Brain Res Bull. 81(1):92-99, 2010. (AS, CE, BE, CC)

It is believed that different electromagnetic fields do have beneficial and harmful biological effects. The aim of the present work was to study the long-term consequences of 50 Hz electromagnetic field (ELF-EMF) exposure with special focus on the development of chronic stress and stress-induced psychopathology. Adult male Sprague-Dawley rats were exposed to ELF-EMF (50 Hz, 0.5 mT) for 5 days, 8h daily (short) or for 4-6 weeks, 24h daily (long). Anxiety was studied in elevated plus maze test, whereas depression-like behavior of the long-treated group was examined in the forced swim test. Some days after behavioral examination, the animals were decapitated among resting conditions and organ weights, blood hormone levels as well as proopiomelanocortin mRNA level from the anterior lobe of the pituitary gland were measured. Both treatments were ineffective on somatic parameters, namely none of the changes characteristic to chronic stress (body weight reduction, thymus involution and adrenal gland hypertrophy) were present. An enhanced blood glucose level was found after prolonged ELF-EMF exposure (p=0.013). The hormonal stress reaction was similar in control and short-term exposed rats, but significant proopiomelanocortin elevation (p<0.000) and depressive-like behavior (enhanced floating time; p=0.006) were found following long-term ELF-EMF exposure. Taken together, long and continuous exposure to relatively high intensity electromagnetic field may count as a mild stress situation and could be a factor in the development of depressive state or metabolic disturbances. Although we should stress that the average intensity of the human exposure is normally much smaller than in the present experiment.

(E) Tasset I, Medina FJ, Jimena I, Agüera E, Gascón F, Feijóo M, Sánchez-López F, Luque E, Peña J, Drucker-Colín R, Túnez I. Neuroprotective effects of extremely low-frequency electromagnetic fields on a Huntington's disease rat model: effects on neurotrophic factors and neuronal density. Neuroscience. 209:54-63, 2012a. (AS, CE, MC, CC, BE, OX, MA, ND)

There is evidence to suggest that the neuroprotective effect of exposure of extremely low-frequency electromagnetic fields (ELF-EMF) may be due, at least in part, to the effect of these fields on neurotrophic factors levels and cell survival, leading to an improvement in behavior. This study was undertaken to investigate the neuroprotective effects of ELFEF in a rat model of 3-nitropropionic acid (3NP)-induced Huntington's disease. Behavior patterns were evaluated, and changes in neurotrophic factor, cell damage, and oxidative stress biomarker levels were monitored in Wistar rats. Rats were given 3NP over four consecutive days (20 mg/kg body weight), whereas ELFEF (60 Hz and 0.7 mT) was applied over 21 days, starting after the last injection of 3NP. Rats treated with 3NP exhibited significantly different behavior in the open field test (OFT) and the forced swim test (FST), and displayed significant differences in neurotrophic factor levels and oxidative stress biomarkers levels, together with a neuronal damage and diminished neuronal density, with respect neuronal controls. ELFEF improved neurological scores, enhanced neurotrophic factor levels, and reduced both oxidative damage and neuronal loss in 3NP-treated rats. ELFEF alleviates 3NP-induced brain injury and prevents loss of neurons in rat striatum, thus showing considerable potential as a therapeutic tool.

(E) Tasset I, Pérez-Herrera A, Medina FJ, Arias-Carrión O, Drucker-Colín R, Túnez I. Extremely low-frequency electromagnetic fields activate the antioxidant pathway Nrf2 in a Huntington's disease-like rat model. Brain Stimul. 2012b Apr 15. [Epub ahead of print] (AS, CE, CC, MA, ND)

Transcranial magnetic stimulation (TMS) is a non-invasive technique used recently to treat different neuropsychiatric and neurodegenerative disorders. Despite its proven value, the mechanisms through which TMS exerts its beneficial action on neuronal function remain unclear. Recent studies have shown that its beneficial effects may be at least partly due to a neuroprotective effect on oxidative and cell damage. This study shows that TMS can modulate the Nrf2 transcriptor factor in a Huntington's disease-like rat model induced by 3-nitropropionic acid (3-NP). Western blot analysis demonstrated that 3-NP caused a reduction in Nrf2 in both cytoplasm and nucleus, while TMS applied to 3-NP-treated rats triggered an increase in cytoplasm and nucleus Nrf2 levels. It was therefore concluded that TMS modulates Nrf2 expression and translocation and that these mechanisms may partly explain the neuroprotective effect of TMS, as well as its antioxidant and cell protection capacity.

(E) Todorović D, Marković T, Prolić Z, Mihajlović S, Rauš S, Nikolić L, Janać B. The influence of static magnetic field (50 mT) on development and motor behaviour of Tenebrio (Insecta, Coleoptera). Int J Radiat Biol. 2012 Aug 1. [Epub ahead of print] (AS, CE, DE, BE)

PURPOSE: There is considerable concern about potential effects associated with exposure to magnetic fields on organisms. Therefore, duration of pupa-adult development and motorbehaviour of adults were analyzed in Tenebrio obscursus and Tenebrio molitor after exposure to static magnetic field (50 mT). MATERIAL AND METHODS: The experimental groups were: control (kept 5 m from the magnets), groups which pupae and adults were placed closer to the North pole, or closer to the South pole of magnetic dipole. The pupae were exposed to the magnetic field until the moment of adult eclosion. The pupa-adult development dynamics were recorded daily. Subsequently, behaviour (distance travelled, average speed and immobility) of adults exposed to the magnetic field was monitored in a circular open field arena. RESULTS: Static magnetic field did not affect pupa-adult developmental dynamic of examined Tenebrio species. Exposure to magnetic field did not significantly change motor behaviour of T. obscurus adults. The changes in the motor behaviour of T. molitor induced by static magnetic field were opposite in two experimental groups developed closer to the North pole or closer to the South pole of magnetic dipole. CONCLUSION: Static magnetic field (50 mT) did not affect on pupa-adult development dynamic of two examined Tenebrio species, but modulated their motor behaviour.

(NE) Türközer Z, Güler G, Seyhan N. Effects of exposure to 50 Hz electric field at different strengths on oxidative stress and antioxidant enzyme activities in the brain tissue of guinea pigs. Int J Radiat Biol. 84(7):581-590, 2008. (AS, CE, OX)

PURPOSE: The aim of this study was to evaluate the possible effects of varied exposure to 50 Hz extremely low frequency (ELF) electric field (EF) on the lipid peroxidation levels and antioxidant enzyme activities in the brain homogenates of guinea pigs. Subjects were exposed to 2 kV/m, 2.5 kV/m, 3 kV/m, 3.5 kV/m, 4 kV/m, 4.5 kV/m and 5 kV/m electric fields for three days, 8 h a day in both vertical and horizontal directions. MATERIALS AND METHODS: Malondialdehyde (MDA), superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were measured in order to identify possible alterations in lipid peroxidation levels and antioxidant status due to electric field exposure. Xanthine oxidase (XO), myeloperoxidase (MPO) and adenosine deaminase (ADA) activities were also evaluated in the same samples. RESULTS: Although the study showed several positive but non-significant findings ($p > 0.05$), we did not find significant differences among all of the exposed groups and sham groups in lipid peroxidation levels and enzyme activities ($p > 0.05$) at all strengths and in both directions. Furthermore, the result was the same when the comparison was made between the groups in vertical directions and horizontal directions ($p > 0.05$). CONCLUSION: The present study observed effects of 50 Hz EF exposure on lipid peroxidation levels and antioxidant defense mechanisms but these were not statistically significant at the 95% confidence level. Further research on the effects ELF-EF exposure on lipid peroxidation levels and antioxidant defence mechanisms are warranted.

(E) van Nierop LE, Slottje P, van Zandvoort MJE, de Vocht F, Kromkout H. Effects of magnetic stray fields from a 7 Tesla MRI scanner on neurocognition: a double-blind randomised crossover study. Occup Environ Med doi:10.1136/oemed-2011-100468 (HU, BE)

Objective: This study characterises neurocognitive domains that are affected by movement-induced time-varying magnetic fields (TVMF) within a static magnetic stray field (SMF) of a 7 Tesla (T) MRI scanner. Methods: Using a double-blind randomised crossover design, 31 healthy volunteers were tested in a sham (0 T), low (0.5 T) and high (1.0 T) SMF exposure condition. Standardised head movements were made before every neurocognitive task to induce TVMF. Results: Of the six tested neurocognitive domains, we demonstrated that attention and concentration were negatively affected when exposed to TVMF within an SMF (varying from 5.0% to 21.1% per Tesla exposure, $p<0.05$), particular in situations were high working memory performance was required. In addition, visuospatial orientation was affected after exposure (46.7% per Tesla exposure, $p=0.05$). Conclusion: Neurocognitive functioning is modulated when exposed to movement-induced TVMF within an SMF of a 7 T MRI scanner. Domains that were affected include attention/concentration and visuospatial orientation. Further studies are needed to better understand the mechanisms and possible practical safety and health implications of these acute neurocognitive effects.

(E) Varró P, Szemerszky R, Bárdos G, Világi I. Changes in synaptic efficacy and seizure susceptibility in rat brain slices following extremely low-frequency electromagnetic field exposure. Bioelectromagnetics. 30(8):631-640, 2009. (AS, CS, FC)

The effects of electromagnetic fields (EMFs) on living organisms are recently a focus of scientific interest, as they may influence everyday life in several ways. Although the neural effects of EMFs have been subject to a considerable number of investigations, the results are difficult to compare since dissimilar exposure protocols have been applied on different preparations or animals. In the present series of experiments, whole rats or excised rat brain slices were exposed to a reference level-intensity (250-500 microT, 50 Hz) EMF in order to examine the effects on the synaptic efficacy in the central nervous system. Electrophysiological investigation was carried out ex vivo, on neocortical and hippocampal slices; basic synaptic functions, short- and long-term plasticity and seizure susceptibility were tested. The most pronounced effect was a decrease in basic synaptic activity in slices treated directly ex vivo observed as a diminution in amplitude of evoked potentials. On the other hand, following whole-body exposure an enhanced short- and long-term synaptic facilitation in hippocampal slices and increased seizure susceptibility in neocortical slices was also observed. However, these effects seem to be transient. We can conclude *that* ELF-EMF exposure exerts significant effects on synaptic activity, but the overall changes may strongly depend on the synaptic structure and neuronal network of the affected region together with the specific spatial parameters and constancy of EMF.

(E) Volkow ND, Tomasi D, Wang GJ, Fowler JS, Telang F, Wang R, Alexoff D, Logan J, Wong C, Pradhan K, Caparelli EC, Ma Y, Jayne M. Effects of low-field magnetic stimulation on brain glucose metabolism. Neuroimage. 51(2):623-628, 2010. (HU, AE, FC)

Echo planar imaging (EPI), the gold standard technique for functional MRI (fMRI), is based on fast magnetic field gradient switching. These time-varying magnetic fields induce electric (E) fields in the brain that could influence neuronal activity; but this has not been tested. Here we assessed the effects of EPI on brain glucose metabolism (marker of brain function) using PET and 18F 2-fluoro-2-deoxy-D-glucose ((18)FDG). Fifteen healthy subjects were in a 4 T magnet during the (18)FDG uptake period twice: with (ON) and without (OFF) EPI gradients pulses along the z-axis (G(z): 23 mT/m; 250 mus rise-time; 920 Hz). The E-field from these EPI pulses is non-homogeneous, increasing linearly from the gradient's isocenter (radial and z directions), which allowed us to assess the correlation between local strength of the E-field and the regional metabolic differences between ON and OFF sessions. Metabolic images were normalized to metabolic activity in the plane positioned at the gradient's isocenter where E=0 for both ON and OFF conditions. Statistical parametric analyses used to identify regions that differed between ON versus OFF ($p<0.05$, corrected) showed that the relative metabolism was lower in areas at the poles of the brain (inferior occipital and frontal and superior parietal cortices) for ON than for OFF, which was also documented with individual region of interest analysis. Moreover the magnitude of the metabolic decrements was significantly correlated with the estimated strength of E ($r=0.68$, $p<0.0001$); the stronger the E-field the larger the decreases. However, we did not detect differences between ON versus OFF conditions on mood ratings nor on absolute whole brain metabolism. This data provides preliminary evidence that EPI sequences may affect neuronal activity and merits further investigation.

(E) Wang X, Liu Y, Lei Y, Zhou D, Fu Y, Che Y, Xu R, Yu H, Hu X, Ma Y. Extremely low-frequency electromagnetic field exposure during chronic morphine treatment strengthens downregulation of dopamine D2 receptors in rat dorsal hippocampus after morphine withdrawal. Neurosci Lett. 433(3):178-82, 2008. (AS, CE, CC)

The aim of this study was to investigate the effect of extremely low-frequency electromagnetic field (ELF-EMF) exposure during morphine treatment on dopamine D2 receptor (D2R) density in the rat dorsal hippocampus following withdrawal. Rats were exposed to ELF-EMF (20 Hz, 14 mT) or sham exposed for 1h per day before injection of morphine (10mg/kg, i.p.) once daily for 12 days. The saline control group was sham exposed for the same period. Immunohistochemistry was used to detect the density of D2Rs on the 1st, 3rd and 5th morphine withdrawal days. The results showed that the density of D2Rs in sham-exposed morphine-treated rats on the 1st and 3rd days of morphine withdrawal was significantly lower than that of the saline control group. The ELF-EMF-exposed morphine group also exhibited a significantly lower density of D2Rs on the 1st and 3rd withdrawal days relative to the sham-exposed morphine group. However, the D2R density in both groups tended to recover as morphine withdrawal days increased. The results suggest that dorsal hippocampal D2Rs are sensitive to morphine withdrawal and that this is potentiated by ELF-EMF pre-exposure during morphine treatment.

(E) Wang X, Zhao K, Wang D, Adams W, Fu Y, Sun H, Liu X, Yu H, Ma Y. Effects of exposure to a 50 Hz sinusoidal magnetic field during the early adolescent period on spatial memory in mice. Bioelectromagnetics. 34(4):275-284, 2013. (AS, CE, BE)

Adolescence is a critical developmental stage during which substantial remodeling occurs in brain areas involved in emotional and learning processes. Although a robust literature on the biological effects of extremely low frequency magnetic fields (ELF-MFs) has been documented, data on the effects of ELF-MF exposure during this period on cognitive functions remain scarce. In this study, early adolescent male mice were exposed from postnatal day (P) 23-35 to a 50 Hz MF at 2 mT for 60 min/day. On P36-45, the potential effects of the MF exposure on spatial memory performance were examined using the Y-maze and Morris water maze tasks. The results showed that the MF exposure did not affect Y-maze performance but improved spatial learning acquisition and memory retention in the water maze task under the present experimental conditions.

(E) Wang Z, Che PL, Du J, Ha B, Yarema KJ. Static magnetic field exposure reproduces cellular effects of the Parkinson's disease drug candidate ZM241385. PLoS One. 5(11):e13883, 2010. (AE, CS, CC)

BACKGROUND: This study was inspired by coalescing evidence that magnetic therapy may be a viable treatment option for certain diseases. This premise is based on the ability of moderate strength fields (i.e., 0.1 to 1 Tesla) to alter the biophysical properties of lipid bilayers and in turn modulate cellular signaling pathways. In particular, previous results from our laboratory (Wang et al., BMC Genomics, 10, 356 (2009)) established that moderate strength static magnetic field (SMF) exposure altered cellular endpoints associated with neuronal function and differentiation. Building on this background, the current paper investigated SMF by focusing on the adenosine A(2A) receptor (A(2A)R) in the PC12 rat adrenal pheochromocytoma cell line that displays metabolic features of Parkinson's disease (PD). **METHODOLOGY AND PRINCIPAL FINDINGS:** SMF reproduced several responses elicited by ZM241385, a selective A(2A)R antagonist, in PC12 cells including altered calcium flux, increased ATP levels, reduced cAMP levels, reduced nitric oxide production, reduced p44/42 MAPK phosphorylation, inhibited proliferation, and reduced iron uptake. SMF also counteracted several PD-relevant endpoints exacerbated by A(2A)R agonist CGS21680 in a manner similar to ZM241385; these include reduction of increased expression of A(2A)R, reversal of altered calcium efflux, dampening of increased adenosine production, reduction of enhanced proliferation and associated p44/42 MAPK phosphorylation, and inhibition of neurite outgrowth. **CONCLUSIONS AND SIGNIFICANCE:** When measured against multiple endpoints, SMF elicited qualitatively similar responses as ZM241385, a PD drug candidate. Provided that the in vitro results presented in this paper apply in vivo, SMF holds promise as an intriguing non-invasive approach to treat PD and potentially other neurological disorders.

(E) Xiong J, He C, Li C, Tan G, Li J, Yu Z, Hu Z, Chen F. Changes of dendritic spine density and morphology in the superficial layers of the medial entorhinal cortex induced by extremely low-frequency magnetic field exposure. PLoS One. 2013 Dec 20; 8(12):e83561. doi: 10.1371/journal.pone.0083561. eCollection 2013. (AS, CE, MC)

In the present study, we investigated the effects of chronic exposure (14 and 28 days) to a 0.5 mT 50 Hz extremely low-frequency magnetic field (ELM) on the dendritic spine density and shape in the superficial layers of the medial entorhinal cortex (MEC). We performed Golgi staining to reveal the dendritic spines of the principal neurons in rats. The results showed that ELM exposure induced a decrease in the spine density in the dendrites of stellate neurons and the basal dendrites of pyramidal neurons at both 14 days and 28 days, which was largely due to the loss of the thin and branched spines. The alteration in the density of mushroom and stubby spines post ELM exposure was cell-type specific. For the stellate neurons, ELM exposure slightly increased the density of stubby spines at 28 days, while it did not affect the density of mushroom spines at the same time. In the basal dendrites of pyramidal neurons, we observed a significant decrease in the mushroom spine density only at the later time point post ELM exposure, while the stubby spine density was reduced at 14 days and partially restored at 28 days post ELM exposure. ELM exposure-induced reduction in the spine density in the apical dendrites of pyramidal neurons was only observed at 28 days, reflecting the distinct vulnerability of spines in the apical and basal dendrites. Considering the changes in spine number and shape are involved in synaptic plasticity and the MEC is a part of neural network that is closely related to learning and memory, these findings may be helpful for explaining the ELM exposure-induced impairment in cognitive functions.

(E) Yi G, Wang J, Wei X, Deng B, Tsang KM, Chan WL, Han C. Effects of extremely low-frequency magnetic fields on the response of a conductance-based neuron model. Int J Neural Syst. 2014 Feb; 24(1):1450007. doi: 10.1142/S0129065714500075. Epub 2013 Dec 11. (CS, AE, EE)

To provide insights into the modulation of neuronal activity by extremely low-frequency (ELF) magnetic field (MF), we present a conductance-based neuron model and introduce ELF sinusoidal MF as an additive voltage input. By analyzing spike times and spiking frequency, it is observed that neuron with distinct spiking patterns exhibits different response properties in the presence of MF exposure. For tonic spiking neuron, the perturbations of MF exposure on spike times is maximized at the harmonics of neuronal intrinsic spiking frequency, while it is maximized at the harmonics of bursting frequency for burst spiking neuron. As MF intensity increases, the perturbations also increase. Compared with tonic spiking, bursting dynamics are less sensitive to the perturbations of ELF MF exposure. Further, ELF MF exposure is more prone to perturb neuronal spike times relative to spiking frequency. Our finding suggests that the resonance may be one of the neural mechanisms underlying the modulatory effects of the low-intensity ELF MFs on neuronal activities. The results highlight the impacts of ELF MFs exposure on neuronal activity from the single cell level, and demonstrate various factors including ELF MF properties and neuronal spiking characteristics could determine the outcome of exposure. These insights into the mechanism of MF exposure may be relevant for the design of multi-intensity magnetic stimulus protocols, and may even contribute to the interpretation of MF effects on the central nervous systems.

(NE) Zhang C, Li Y, Wang C, Lv R, Song T. Extremely low-frequency magnetic exposure appears to have no effect on pathogenesis of Alzheimer's disease in aluminum-overloaded rat. PLoS One. 2013 Aug 12;8(8):e71087. doi: 10.1371/journal.pone.0071087. eCollection 2013. (AS, CE, BE, MC, ND)

OBJECTIVE: Extremely low-frequency magnetic field (ELF-MF) has been reported to be of potential pathogenetic relevance to Alzheimer's disease (AD) for years. However, evidence confirming this function remains inconclusive. Chronic Al treatment has been identified as a contributing factor to cognitive function impairment in AD. This study aims to examine whether or not ELF-MF and Al have synergistic effects toward AD pathogenesis by investigating the effects of ELF-MF with or without chronic Al treatment on SD rats. METHODS: Sprague-Dawley (SD) rats were subjected one of the following treatments: sham (control group), oral Al (Al group), ELF-MF (100 µT at 50 Hz) with oral Al (MF+Al group), or ELF-MF (100 µT at 50 Hz) without oral Al (MF group). RESULTS: After 12 wk of treatment, oral Al treatment groups (Al and MF+Al groups) showed learning and memory impairment as well as morphological hallmarks, including neuronal cell loss and high density of amyloid-β (Aβ) in the hippocampus and cerebral cortex. ELF-MF without Al treatment showed no significant effect on AD pathogenesis. ELF-MF+Al treatment induced no more damage than Al treatment did. CONCLUSIONS: Our results showed no evidence of any association between ELF-MF exposure (100 µT at 50 Hz) and AD, and ELF-MF exposure does not influence the pathogenesis of AD induced by Al overload.

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 10

Effects of Electromagnetic Fields From Wireless Communication upon the Blood-Brain Barrier

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I. INTRODUCTION

The Blood-Brain Barrier

Some organs of crucial importance for the function of our bodies are protected from exposure to potentially harmful compounds in the blood. Thus the brain, the eyes (which are protrusions of the brain), the testes and the follicles of the ovaries have special barriers between the capillaries and the tissue. In the normal brain, the passage of compounds over this barrier, the Blood-Brain Barrier (BBB), is highly restricted.

The BBB is a hydrophobic barrier formed by the vascular endothelial cells of the capillaries in the brain with tight junctions between them leaving no openings between the vessel lumen and the surrounding brain. The existence of the mammalian BBB was discovered in the late 19th century by the German bacteriologist Paul Ehrlich and his student, Edwin Goldman. Paul Ehrlich found, that when he injected dyes into the systemic blood circulation, the brain tissue did not take up any of the stain. A barrier surrounding the brain tissue at the site of the brain micro vessels seemed to be a logic explanation to these findings.

There is scientific evidence that the BBB exists not only in vertebrates, but also in insects (1), crustaceans and cephalopod molluscs (such as the cuttlefish) (2) and in elasmobranchs (cartilaginous fishes such as sharks) (3) and helices (landsnails) (4), maintaining ionic integrity of the neuronal bathing fluid.

The BBB seems to be present very early in the foetal development. Also, at an early stage, there seems to be a cerebrospinal fluid barrier, which excludes cerebrospinal fluid (CST) protein from the brain extracellular space (5).

BBB Anatomy and Physiology

The tight junctions of the BBB are composed of tight junction proteins (occludin, claudin and zonula occludens, where the zonula occludens is the intracellular peripheral membrane protein that anchors claudin and occludin to the actin cytoskeleton (6). An important part is the binding of claudin proteins on opposing membranes, where claudin-5 in particular is crucial in the BBB (7). Astrocytes are surrounding the outer surface of the endothelial cells with protrusions, called end feet, and are implicated in the maintenance, functional regulation and repair of the BBB. The astrocytes form a connection between the endothelium and the neurons and constitute a second barrier to hydrophilic molecules (see Figure 1).

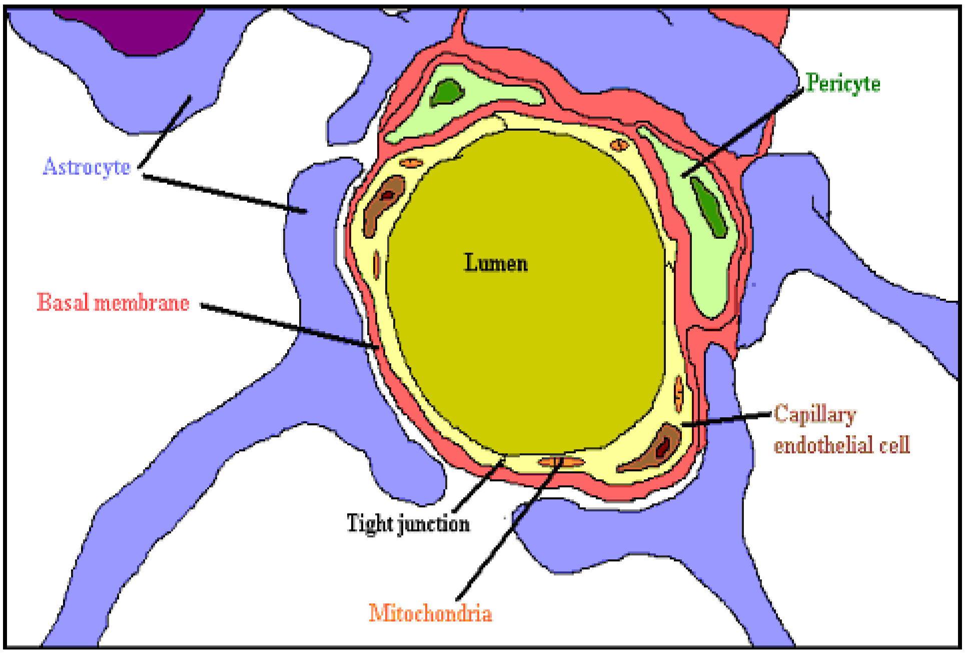


Fig. 1. The mammalian BBB

Other periendothelial accessory structures of the BBB include pericytes and a bilayer basal membrane which surrounds the endothelial cells and pericytes. The basement membrane (basal lamina) supports the ablumenal surface of the endothelium and may act as a barrier to passage of macromolecules. The pericytes are a type of macrophages, expressing macrophage markers with capacity for phagocytosis but also for antigen presentation. In fact, the pericytes, which cover about 25% o the capillary surface (8), seem to be in a position to significantly contribute to central nervous system (CNS) immune mechanisms (9). The pericytes also have other functional roles: with their capability for contractility they seem to serve as a smooth muscle equivalent, and through regulation of endothelial cells they maintain the stability of blood vessels (9). Additionally, the pericytes seem to be highly involved in many diseases, both infectious and autoimmune, and also in other diseases such as Alzheimer’s by production of amyloid. Also, by regulating their vascular permeability, the pericytes are supposed to play an important role in inflammatory diseases (9).

Physiologically, the microvasculature of the central nervous system (CNS) differs from that of peripheral organs. It is characterized not only by its tight junctions, which seal cell-to-cell contacts between adjacent endothelial cells, but also by the low number of pinocytotic vesicles for nutrient transport through the endothelial cytoplasm and its lack of fenestrations, and the five-fold higher number of mitochondria in BBB endothelial cells compared to muscular endothelia in rat (10). All this speaks in favour of an energy-dependent transcapillary transport. These above-described membrane properties of the BBB control the bidirectional exchange of molecules between the general circulation and the central nervous system. By at least four mechanisms, the endothelial cells directly control the flux of solutes into the brain parenchyma. Firstly, the tight junctions and low number of pinocytotic vesicles guarantee that proteins cannot pass freely into the brain parenchyma.
Secondly, solutes which are not highly lipid soluble, or which do not bind to selective transporters with high affinity, are excluded from free exchange. By means of this lipid solubility, carbon dioxide and oxygen, among many others, are able to enter the brain interstitial fluid passively, whereas the passage of, for example sugars and many amino acids, depends on other, active mechanisms. Thirdly, the BBB has a capacity to metabolize certain solutes, such as drugs and nutrients (11). Fourthly, active transporters maintain the levels of certain solutes at specific values within the brain interstitial fluid, made possible by active transport against the concentration gradients. These enzyme systems are differently distributed between the luminal and the ablumenal membranes of the endothelial cells, thus gaining the BBB polarity properties. For example, Na^+-K^+-ATPase is located on the antilumenal membrane (12).

It has been proposed that the active transport across the brain capillaries might be the most important mechanism for the regulation of the internal milieu within the brain parenchyma. Also, it has been proposed that this mechanism, requiring energy to function properly, might be the one most sensitive to disease and that interference with this active transport could play an important part in the neurological dysfunction seen in many metabolic disorders (12).

It is important to have information on possible differences between homo and other mammals. The mammalian brain at large seems to have a uniform anatomy of its BBB constituents preserved through the evolution, and very little information about differences between mammalian species has been available. However, recently very interesting observations have been published. Humans have evolved protoplasmic astrocytes that are both larger (27-fold greater volume) and far more elaborate than their rodent counterparts. These astrocytes reside near blood vessels, and their processes contribute to the BBB (13). When the end feet of human and rodent protoplasmic astrocytes are compared, it is shown that nearly all astrocytes in both species contact the vasculature, but in the human brain, the end feet completely encompass the vessels while the rodent astrocytes form rosettes of end feet around the vasculature. The number of mithochondria is however equally abundant in human and rodent end feet (14).

Comparisons between mammalian species concerning enzymatic functions in the BBB are few in number. Similarities are described: mouse *vs* human (15) and rat *vs* human (16), while differences are demonstrated between rodent and dog BBB leading to the conclusion that the canine BBB may be preferable to that of the rat as a model for studies of glucose transport relevant to human brain (17).

In summary, the BBB serves as a regulatory system that stabilizes and optimizes the fluid environment of the brain's intracellular compartment (18-20). The intact BBB protects the brain from damage, whereas the dysfunctioning BBB allows influx of normally excluded hydrophilic molecules into the brain tissue. This might lead to cerebral oedema, increased intracranial pressure, and in the worst case, irreversible brain damage.

II. DISRUPTION OF THE BLOOD-BRAIN BARRIER

The normal selective permeability of the BBB can be altered in several pathological conditions such as epileptic seizures (21) or extreme hypertension (22)and also transient openings of the BBB might lead to permanent tissue damage (22). Considering the ensuing leakage of substances from the blood circulation into the brain tissue, harmful substances might disrupt the cellular balance in the brain tissue and in the worst case, even carcinogenic substances might pass into the brain tissue. It has also been shown that an increased permeability of the BBB is seen in cases of oxidative stress (23), where BBB dysfunction and neurodegeneration were shown to be mediated through an excitotoxicity mechanism by the serine protease tissue plasminogen activator, with NO and $ONOO^-$ as downstream mediators (23).

Opening of the BBB thus can have detrimental effects and since it has been shown for a few decades that EMFs have the potency to increase the permeability of this barrier, a major debate is going on in society with increasing intensity. In the following, we try to clarify the actual status of the available evidence in the field.

Early Studies

In early studies on the effects of low-intensity EMFs on the BBB, various compounds were injected intravenously, followed by EMF exposure and comparisons of the penetration into the brain tissue between sham and exposed animals.

Frey et al. (25) found increases in the BBB permeability of rats to fluorescein after 30 min of exposure to both pulsed and continuous waves (CWs) at 1.2GHz with average power densities of $0.2 mW/cm^2$. Similar observations were made in a study with 180 animals by Oscar and Hawkins (26). Exposure of anaesthetized rats for 20 min to 1.3GHz of pulsed EMFs with average power densities of $0.3 mW/cm^2$ resulted in leakage of 14C-mannitol, dextran, and inulin into the cerebellar brain tissue, as well as inulin and dextran leakage from capillaries into hypothalamic and medullar tissue. Also, BBB permeability to mannitol was investigated in un-anaesthetised rats, which were exposed to pulsed radiation or sham exposed for 20 min. The animals were sacrificed at different time intervals after the exposure. BBB permeability was seen in the groups sacrificed 8 min and 4 h after exposure, but to a much lesser extent in those sacrificed after 8 h. Finally, the permeation of mannitol through the BBB was found to be a very definite function of exposure parameters such as power density, pulse width, and the number of pulses per second. However, in later studies, Oscar et al. (27) emphasised that changes of BBB permeability after microwave exposure partly could be explained by an increase of local cerebral blood flow. In accordance with this, they concluded that their initial findings (26) might be of less magnitude than originally thought (Table 1).

Effects of Radiofrequency/Microwave Radiation upon the BBB – A summary of Previous Studies

Table 1. BBB permeability after EMF exposure. (From Nittby et al. (24))

Reference	EMF Frequency (MHz)	Modulation, pulses per second (pps)	Duration of exposure	SAR (W/kg)	Effect on BBB permeability?	Total number of animals included in the study	Tracer or studied effect	Remark
Findings by the Lund Group								
Salford et al. 1994	915	CW and pulse-modulated with repetition rates of 8, 16, 50 and 200 /s	2 hours	0.016-5 W/kg	Yes	246 Fischer 344 rats	Albumin extravasation	
Persson et al. 1997	915	217, 50 Hz and CW	2-960 min	0.0004-0.95 W/kg	Yes	1002 Fischer	Albumin extravasation	

				average whole-body		344 rats		
Salford et al. 2003	915	GSM	2 hours	0.002-0.2 W/kg	Yes		Albumin extravasation and dark neurons	Effect was seen 50 days after the exposure
Eberhardt et al. 2008	915	GSM	2 hours	0.0002-0.2 W/kg	Yes	96 Fischer 344 rats	Albumin extravasation and dark neurons	Albumin extravasation 14 days after exposure, dark neurons 28 days after exposure
Mobile phone exposure								
Fritze et al. 1997	900	GSM	4 hours	0.3 to 7.5 W/kg	Yes		Albumin	Albumin extravasation only reported for SAR-values of 7.5 W/kg
Töre et al. 2001	900	GSM	2 hours	0.12; 0.5 and 2.0 W/kg	Yes	70 Sprague-Dawley	Albumin leakage, seen with fluorescein-labelled proteins	Albumin extravasation at SAR-values

								of 0.5 and 2.0 W/kg
Neubauer et al. 1990	2450	100 pps	30-120 min	Average 2 W/kg	Yes		Rhodamine-ferritin complex	No leakage at 1 W/kg at short-term exposure of 15 min
Tsurita et al. 2000	1439	TDMA	1 hour daily, for 2 or 4 weeks	Average whole-body 0.25 W/kg; peak in the brain of 2 W/kg	No	36 Sprague-Dawley rats	Evans blue, albumin	
Kuribayashi et al. 2005	1439	TDMA, 50 pps	90 min daily, for 1 to 2 weeks	Average brain power densities of 2 or 6 W/kg; average whole-body 0.29 or 0.87 W/kg	No	40 Fischer 344 rats	Three BBB-related genes; FICT-dextran and albumin extravasation	

Finnie et al. 2001	898.4	GSM	1 hour	Whole-body of 4 W/kg	No	60 mice	Albumin extravasation	
Finnie et al. 2002	900	GSM	1 hour daily, 5 days a week for 104 weeks	Average whole-body 0.25; 1.0; 2.0 and 4.0 W/kg	No	207 mice	Albumin extravasation	
Franke et al. 2005b	1800	GSM	1 to 5 days	Average 0.3 W/kg	No		Sucrose permeation	In vitro model of BBB
Schirmacher et al. 2000	1800	GSM	4 days	Average 0.3 W/kg	No		Sucrose permeation	In vitro model of BBB
Franke et al. 2005a	1966	UMTS	1 to 3 days	Average 1.8 W/kg	No		Sucrose and albumin permeation	In vitro model of BBB
Cosquer et al. 2005	2450	500 pps	45 min	Average whole-body 2 W/kg	No	Rats	Scopolamine methylbromide extravasation	Indirect investigation of BBB opening by performance in radial arm maze

RF exposure of other kinds								
Frey et al. 1975	1200	1000 pps and CW	30 min	0.2 mW/cm^2	Yes	Rats	Fluorescein	
Oscar and Hawksins 1977	1300	50-1000 pps	20 min	0.3 mW/cm^2	Yes	180 Wistar rats	Leakage of mannitol, dextran and inulin	
Preston et al. 1979	2450	CW	30 min	0.1 – 30 mW/ cm^2	No	Rats	Mannitol	
Merritt et al. 1978	1200 and 1300	1000 pps and CW	30 min	2-75 mW/ cm^2 and 0.1-50 mW/cm^2	No	Sprague Dawley rats	Fluorescein, mannitol, serotonin	Tried to replicate findings by Frey et al. (1975) and Oscar and Hawkins (1977)
Ward et al. 1982	2450	CW	30 min	10-30 mW/ cm^2	No	Rats	Sucrose and inulin	
Ward and Ali 1985	1700	CW and 1000 pps	30 min	0.1 W/kg	No	Rats	Sucrose and inulin	
Albert and	2450	CW	2 hours	2.5 W/kg	Yes	80 Chinese	Horseradish peroxidase	Reversible

Kerns 1981						hamsters		process with no HRP permeation after 1-2 recovery
Gruenau et al 1982	2800	CW and 500 pps	30 min	1-40 mW/cm^2	No	31 rats	Sucrose	
Lin and Lin 1980	2450	500	20 min	0.04-80 W/kg	No	Wistar rats	Evans blue and sodium fluorescein	
Lin and Lin 1982	2450	25-500	5-20 min	0.04-240 W/kg	No	51 Wistar rats	Evans blue	BBB permeability only at SAR of 240 W/kg, which is a thermal effect
Goldman et al. 1984	2450	500		240 W/kg	No		Rubidium-86	Hyperthermia induced BBB permeability
Williams et al. 1984a	2450	CW	30-180 min	4-13 W/kg	No	32 Fischer 344 rats	Fluorescein	BBB permeability only at

								hyperthermic levels > 41°C
Williams et al. 1984b	2450	CW	30-180 min	4-13 W/kg	No	20 Fischer 344 rats	HRP	
Williams et al. 1984c	2450	CW	30-90 min	13 W/kg	No	24 Fischer 344 rats	Sucrose	
Williams et al. 1984d	2450	CW	30-180 min	4-13 W/kg	No	66 Fischer 344 rats	Fluorescein, HRP, sucrose	BBB permeability only at brain temperatures > 40°C
Quock et al. 1986	2450	CW	10 min	24 W/kg		Mice	Domperidone	BBB permeability due to temperature increase
Quock et al. 1987	2450	CW	10 min	24 W/kg		Mice	Domperidone	BBB permeability due to temperature increase

Moriyama et al. 1991	2450	CW				21 Sprague Dawley rats	HRP	BBB permeability due to temperature increase
Nakagawa et al. 1994	2450	CW				Japanese monkeys		BBB permeability due to temperature increase
MRI exposure				**Magnetic field**				
Shivers et al. 1987			23 min	0.15 T static magnetic field	Yes		HRP	Standard MRI procedure
Preston et al. 1989			23 min	4.7 T static magnetic field	No	Rats	Sucrose	Standard MRI procedure
Prato et al.	65		23 min x 2	0.15 T static	Yes	43	Diethylenetriaminepentaacetic	Standard MRI

1990			magnetic field		Sprague Dawley rats	acid (DTPA)	procedure
Prato et al. 1994		23 min x 2	1.5 T static magnetic field	Yes	50 rats		Standard MRI procedure
Garber et al. 1989			0.3-0.5 T static magnetic field	Yes	Rats	Mannitol	Standard MRI procedure
Adzamli et al. 1989				No			Standard MRI procedure
ELF exposure							
Öztas et al. 2004	50	8 hours daily for 21 days	0.005T	Yes	34 Wistar rats	Evans-blue	BBB disruption in diabetic rats, but not in normoglycemic rats

In an attempt to repeat the findings of Oscar and Hawkins (26), Preston et al. (28) found no increase in the uptake of 14C-mannitol in anaesthetised rats after 2450MHz CW exposure for 30 min at power densities of 0.1 to 30mW/cm^2. Preston et al. further concluded that the increased BBB permeability, which had been observed by Oscar and Hawkins (26) in cerebellum and medulla, possibly had been misinterpreted and was not due to the EMF exposure. Rather, changes in blood flow and water influx or egress were supposed to be responsible for the BBB permeability in these caudal parts of the brain. Also, further attempts, made by Merritt et al. (1978) (29), to replicate the findings of Oscar and Hawkins from 1977, resulted in the conclusion that no repetition of the initial findings could be made. Merritt et al. (29) tried to replicate also the findings of Frey et al. (25), but reported that no changes were seen.

However, Frey commented upon this in an article in 1998, where he pointed out that, in fact, statistical analysis by the editor and reviewer of the data from the study by Merritt et al. provided a confirmation of the findings of Frey et al. (25) (30).

No alteration of BBB permeation of 14C-sucrose and 3H-inulin was found by Ward et al. (31)after exposure of anaesthetised rats to CW at 2450MHz for 30 min at power densities of 0, 10, 20, or 30 mW/cm^2 after correction for thermal effects. Similarly, Ward and Ali (32) observed no permeation after 1.7GHz exposure at SAR of 0.1 W/kg, using the same exposure duration and injected tracers as Ward et al. (31). Absence of EMF induced BBB permeability was also reported by Gruenau et al. (33), after injection of 14C-sucrose in conscious rats and exposure 30 min pulsed energy (2.8GHz at 0, 1, 5, 10, or 15mW/cm^2) or continuous wave (2.8 GHz, 0, 10, or 40 mW/cm^2).

Proof of EMF-induced BBB permeability was put forward by Albert and Kerns (34), who exposed un-anaesthetised Chinese hamsters to 2,450MHz CWs for 2 h at SARs of 2.5 W/kg. In one-third of the exposed animals there was an increased permeability of the BBB to horseradish peroxidase (HRP) and the endothelial cells of these irradiated animals had a 2–3-fold higher number of pinocytotic vesicles with HRP than the sham animals. The mechanism of BBB permeability seemed to be reversible, since animals allowed to recover for 1 or 2 h after the EMF exposure had almost no HRP permeation. A total number of 80 animals were included in this study.

Temperature Dependence

In further studies, more attention was directed towards the effects of hyperthermia, resulting from exposure at high SAR-levels, on BBB permeability.

A study correlating changes of BBB permeability with the quantity of absorbed microwave energy by Lin and Lin (35), using Evans blue and sodium fluorescein as indicators of BBB permeation, showed that 20 min of 2,450MHz exposure of anaesthetised Wistar rats caused no alteration of BBB permeability even at SAR values of 80 W/kg. Notably, the same lack of alteration was observed also at lower SAR-values, down to 0.04 W/kg. In further studies by the same group (36), no permeation of Evans blue could be observed after exposure to 2,450MHzB RFs for 5–20 min when the SAR-values ranged from 0.04–200 W/kg. Not until a SAR-value of 240 W/kg, with ensuing rise in brain temperature to 43°C, was applied, the BBB permeability increased. These observations of demonstrable increases of BBB permeability associated with intense, microwave-induced hyperthermia were supported by another study by the same group (37).

In a series of EMF exposures at 2,450MHz CW, Williams et al. (38-40) concluded that increase of BBB permeability might not be explained by microwave exposure, but rather temperature increases and technically derived artefacts such as increase of the cerebral blood volume and a reduction in renal excretion of the tracer. Significantly elevated levels of sodium fluorescein (38) were found only in the brains of conscious rats made considerably hyperthermic by exposure to ambient heat for 90 min or 2,450MHz CW microwave energy for 30 or 90 min, but this was at high SAR values, 13 W/kg—far beyond the ICNIRP limit of 2 W/kg (41) —and not comparable to the experiments performed by, among others, our group, as described below.

With more research into the area of EMF induced BBB permeability, it became evident that with high-intensity EMF exposure resulting in tissue heating, the BBB permeability is temperature dependent (42). Thus, the importance of differentiating between thermal and non-thermal effects on the integrity of the BBB was realized. This is the reason why studies with increases of BBB permeability due to exposure to SAR-values well above recommended exposure levels (43-46) need to be considered from another point of view, as compared to those focusing on the non-thermal effects of EMFs.

Continued Studies—MRI and BBB Permeability

Following the increasing use of magnetic resonance imaging (MRI), the effects of MRI radiation upon BBB permeability were investigated more thoroughly. MRI entails the concurrent exposure of subjects to a high-intensity static field, a radiofrequency field, and time-varying magnetic field. Shivers et al. (47) observed that exposure to a short (23 min) standard (of those days) clinical MRI procedure at 0.15 Tesla (T) temporarily increased the permeability of the BBB to horseradish peroxidase (HRP) in anaesthetised rats. This was revealed by electron microscopy (EM), to be due to an amplified vesicle-mediated transport of HRP across the microvessel endothelium, to the ablumenal basal lamina and extracellular compartment of the brain parenchyma. This vesicle-mediated transport also included transendothelial channels. However, no passage of the tracer through disrupted interendothelial tight junctions was present.

During the next few years, more groups studied the effects of MRI exposure on the BBB permeability by injection of radioactive tracers into rats. One supported (48)while others contradicted (49, 50) the initial findings made by Shivers et al. (47). Garber et al. exposed rats to MRI procedures at 1.5, 0.5, and 0.3 T with RFs of 13, 21, and 64 MHz, respectively (48). Brain mannitol concentration was significantly increased at 0.3 T and 0.5 T but not at 1.5 T. No decrease in plasma mannitol concentration of MRI exposed animals was found and thus the authors concluded that effects of MRI associated energies on mannitol transport do not occur measurably in the body, and might be more specific to brain vasculature. Preston et al. (50) found no significant permeation of blood-borne 14C-sucrose into brain parenchyma in anesthetized rats subjected to 23 min of MRI at 4.7 T and RFs at 12.5 kHz. However, the authors pointed out that if the MRI effect was focal and excess tracer counts were found only in restricted sites, there could have been MRI induced extravasation of sucrose that was not detected, due to the preponderance of normal tissue counts. When Preston et al. (50) compared the lack of BBB leakage in their study to the MRI induced leakage which had been observed by Shivers et al. (47), they also concluded that certain characteristics of electric and magnetic fields, which were present in the study by Shivers et al. but not in their own work, could have been critical to the observed effects.

In 1990, further studies by the Shivers-Prato group were presented (51) and the group could now quantitatively support its initial findings, in a series of 43 Sprague-Dawley rats. The BBB permeability to diethylenetriaminepentaacetic acid (DTPA) increased in rats after two sequential 23 min MRI exposures at 0.15 T. It was suggested that the increased BBB permeability could result from a time-varying magnetic field mediated stimulation of endocytosis. Also, the increased BBB permeability could be explained by exposure-induced increases of intracellular Ca^{2+} in the vascular endothelial cells. Since the Ca^{2+} is an intracellular mediator, increases of BBB permeability could possibly be initiated in this way. A few years later, in a series of 50 rats, the Shivers - Prato group also found that the BBB permeability in rats is also altered by exposure to MRI at 1.5T for 23 min in 2 subsequent exposure sessions (52).

Studies by the Lund Group

Two of us found these observations highly interesting:

- the neurosurgeon (LGS) in the hope to utilize possible applications of EMF to make the blood-brain barrier (BBB) more penetrable to chemotherapy, in order to treat brain cancers more effectively. An intact BBB keeps out chemotherapy agents, allowing cancer cells to hide behind the BBB.

- the radiophysicist (BRRP) interested in possible adverse effects of the MRI technique.

After a visit to Shivers' group in London Ontario in 1988, we started work in Lund in 1988, studying the effects of MRI on rat brain and we found, by the use of Evans Blue, the same increased permeability over BBB for albumin (53).

This work was continued by separating the constituents of the MRI field: RF, undulant magnetic field, and static magnetic field. Since RF turned out to be the most efficient component of the MRI, the following studies focused mainly on the RF effects. Striving for investigating the actual real-life situation, endogenous substances, which naturally circulate in the vessels of the animals, were used. In line with this, albumin and also fibrinogen leakage over the BBB were followed after identification of albumin with rabbit antibodies (see Figure 2 and 3) and rabbit anti-human fibrinogen.

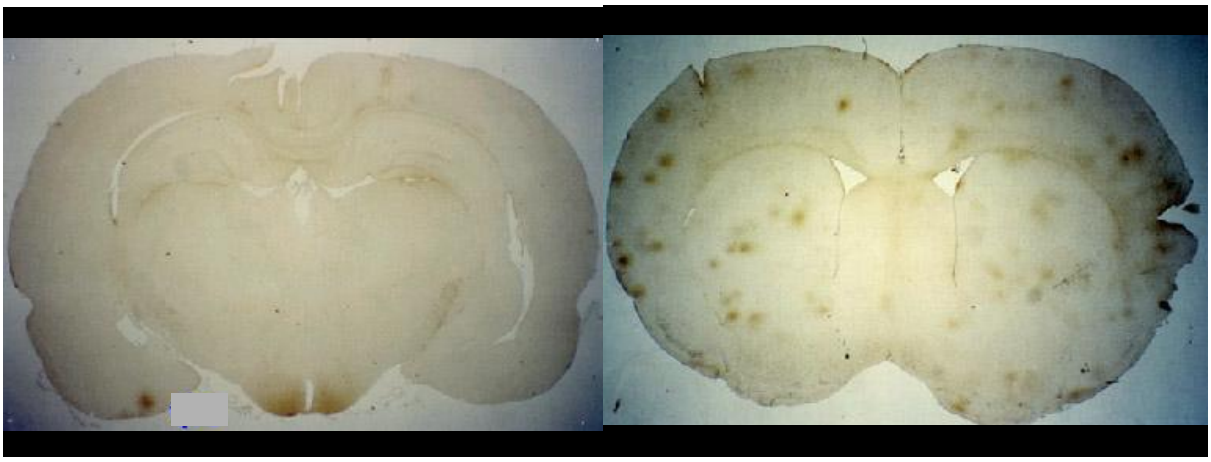


Figure 2. Albumin extravasation in rat brain (material from Persson et al. 1997)(54).
Left: control brain with albumin staining in hypothalamus, which serves as an inbuilt-control of the staining method, since the hypothalamus lacks BBB, and one occasional staining.
Right: Brain of EMF exposed rat, with multiple albumin positive foci.

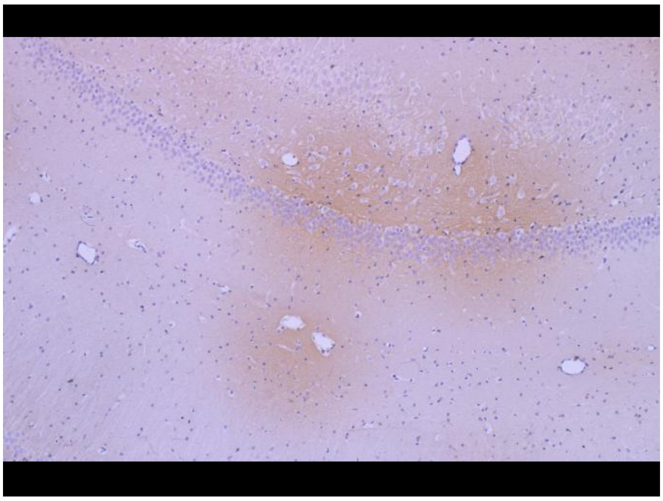


Figure 3. Albumin extravasation around vessels in the brain of an EMF exposed rat.

The work by Blackman et al. (55, 56) ~~made the ground~~ laid the groundwork for studies on the frequency modulation 16 Hz and its ~~harmonies~~ harmonics 4 and 8 Hz. A carrier wave of 915 MHz was used. At the suggestion of Östen Mäkitalo (Telia), a pioneer in mobile phone development, who introduced 50 Hz (DUX) and 217 Hz (GSM) modulation in new digital wireless communication systems, we also included theses frequencies. This paralleled the first BBB study results that were published in 1992-1994 (57-59).

The result of our continued work, comprising more than 1000 animals, with exposure to both CWs and pulsed modulated waves, in the most cases lasting for 2 h, showed that there was a significant difference between the amount of albumin extravasation in the exposed animals as compared to the controls. In the exposed group 35–50% of the animals had a disrupted BBB as seen by the amount of albumin leakage, while the corresponding leakage in the sham exposed animals was only 17% (for results see Figure 4) (54).

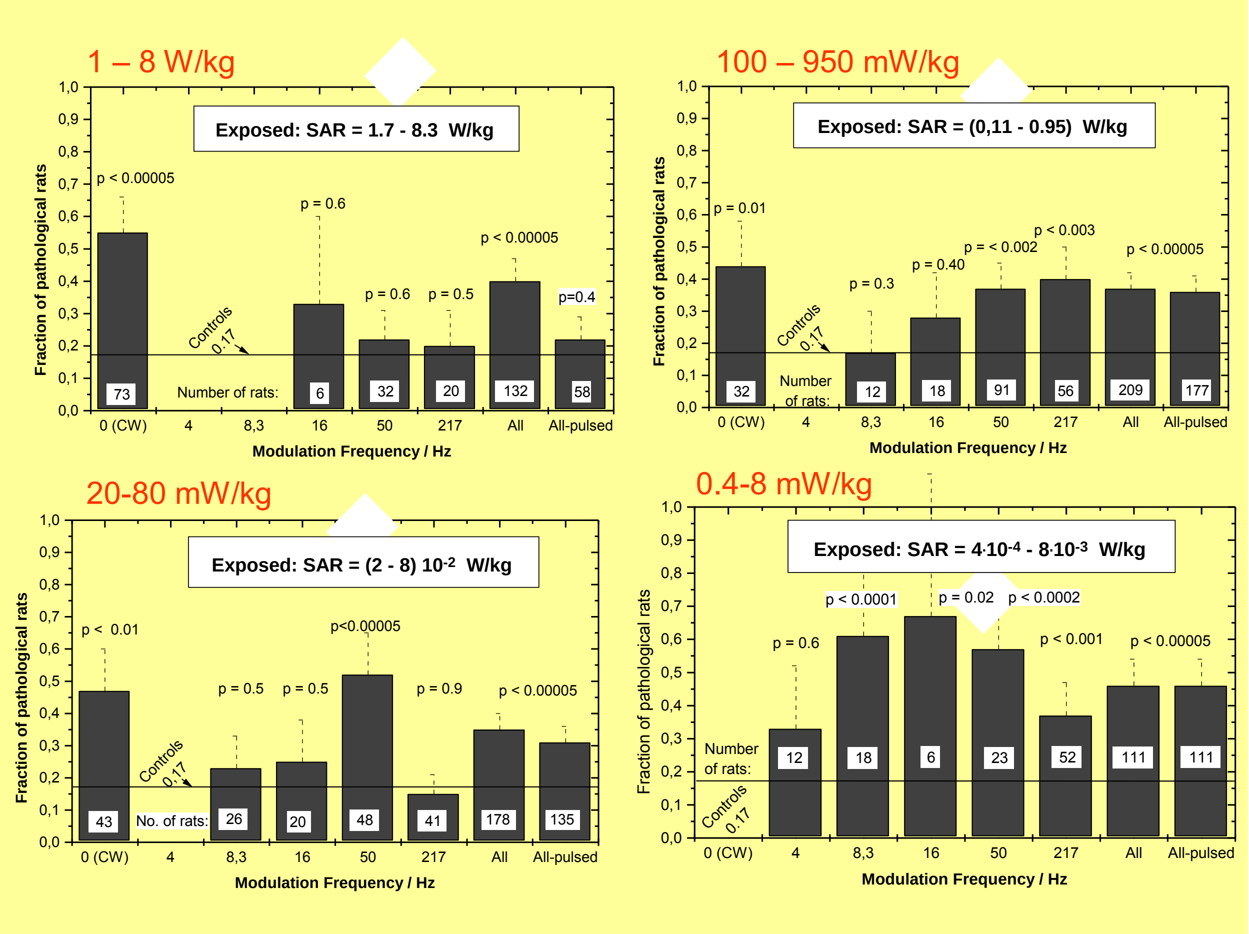


Figure 4. Albumin extravasation score as a result of EMF exposure (results from the study by Persson et al. (54)).

The fact that sham-exposed control animals also show some amount of albumin extravasation (see Figure 4), is most likely due to our very sensitive methods for immune histological examination. However, it is hard to explain the fact that although all animals in the 1997 series were inbred Fischer 344 rats, only every second animal, at the most, showed albumin leakage after EMF exposure. The question, what might protect the remaining 50% of the exposed animals from BBB disruption, is highly intriguing. It should be noted that in our large series, only in one single animal fibrinogen leakage has been observed (54).

Another conclusion from the 1997 study is that the number of pathological leakages in exposed animals is more frequent, and also more severe, per animal compared to the controls. This is an interesting observation as the prevailing opinion is that pulse modulated electromagnetic fields are more potent in causing biological effects.

In a statistical re-evaluation of our material published in 1997, where only exposed rats with a matched unexposed control rat are included, we found for the most interesting modulation frequency 217 Hz, i.e. that of GSM, that at SAR-values of 0.2 to 4 mW/kg 48 exposed rats had a significantly increased albumin leakage ($p < 0.001$) as compared their 48 matched controls. On the other hand, SAR-values of 25-50 mW/kg, gave no significant difference between 22 exposed rats *vs* their matched controls (Wilcoxon´s Rank Test, 2-sided p-value) (60).

In all our earlier studies we showed albumin extravasation immediately after exposure as described above. In later years we have performed a series of experiments where the animals were allowed to survive for 7 days (61), 14 days, 28 days (62) or 50 days (63) after one single 2-hour exposure to the radiation from a GSM mobile phone. All were exposed in TEM-cells to a 915 MHz carrier wave as described below. The peak power output from the GSM mobile phone fed into the TEM-cells was 1 mW, 10 mW, 100 mW and 1000 mW per cell respectively for the 7-14-28-days survival animals, resulting in average whole-body SAR of 0.12 mW/kg, 1.2 mW/kg, 12 mW/kg and 120 mW/kg for four different exposure groups SAR-values of 2, 20 and 200 mW/kg mW/kg for 2 hours for the 50-days survival animals.

Albumin extravasation over the BBB after GSM exposure seemed to be time-dependent, with significantly increased albumin in the brain parenchyma of the rats, which had survived for 7 and 14 days, but not for those surviving 28 days. After 50 days, albumin extravasation was significantly increased again, with albumin-positive foci around the finer blood vessels in white and gray matter of the exposed animals.

In connection to the albumin passage over the BBB, albumin also spread in the surrounding brain tissue. A significantly increased uptake of albumin in the cytoplasm of neurons could be seen in the GSM exposed animals surviving 7 and 14 days after exposure, but not in those surviving 28 or 50 days.

Neuronal uptake

Extravasated albumin rapidly diffused down to, and beyond, concentrations possible to demonstrate accurately immunohistologically. However, the initial albumin leakage into the brain tissue (seen within hours in ~40% of exposed animals in our previous studies) most likely started a vicious circle of further BBB opening.

It has been postulated that albumin is the most likely neurotoxin in serum (64). Hassel et al. (65) have demonstrated that injection of albumin into the brain parenchyma of rats gives rise to neuronal damage. When 25 µl of rat albumin is infused into rat neostriatum, 10 and 30, but not 3 mg/ml albumin causes neuronal cell death and axonal severe damage. It also causes leakage of endogenous albumin in and around the area of neuronal damage. Albumin in the dose 10 mg/ml is approximately equivalent to 25% of the serum concentration.
It is less likely that the albumin leakage demonstrated in our experiments locally reaches such concentrations. However, we have seen that in the animals surviving 28 and 50 days after 2 hours of GSM exposure, there was a significantly increased incidence of neuronal damage as compared to the sham controls. In the 7-days and 14-days survival animals, on the other hand, no such increase of neuronal damage was seen.

In the 50-days post-exposure survival study, a 2 h exposure to GSM at SAR values 200, 20, and 2 mW/kg resulted in a significant ($p = 0.002$) neuronal damage in rat brains of the exposed animals as compared to the controls 50 days after the exposure occasion (Salford et al., 2003)(63). We have followed up this observation, as mentioned above, in a study where 96 animals were sacrificed 14 and 28 days respectively after an exposure for 2 h to GSM mobile phone electromagnetic fields at SAR values 0 (controls), 0.12, 1.2, 12 and 120 mW/kg. Significant neuronal damage is seen after 28 days and albumin leakage after 14. Our findings may support the hypothesis that albumin leakage into the brain is the cause for the neuronal damage observed after 28 and 50 days (62).

The damaged neurons in the above mentioned studies took the shape of so-called dark neurons. Three main characteristics of the damaged dark neurons have been proposed (66): (i) irregular cellular outlines, (ii) increased chromatin density in the nucleus and cytoplasm and (iii) intensely and homogenously stained nucleus. The damaged dark neurons found in the 50 days-survival animals were investigated regarding signs of apoptotic markers, but we found no positive staining for Caspase-3, a marker for apoptosis (Bexell et al. unpublished results). However, the albumin leakage out in the neuropil in connection to EMF exposure might start other deleterious processes, leading to the formation of the dark neurons.

A group in Turkey performed similar experiments. However, also the presumed protective effects of the antioxidant Ginko biloba (Gb) were examined by Ilhan et al. (67). About 22 female Wistar rats were exposed to a 900 MHz electromagnetic GSM near-field signal for 1 h a day for 7 days. In the GSM only group, the pathological examination revealed scattered and grouped dark neurons in all locations, but especially in the cortex, hippocampus and basal ganglia, mixed in among normal neurons. A combined non-parametric test for the four groups revealed that the distributions of scores differed significantly between the control and the GSM only exposure group ($p < 0.01$).

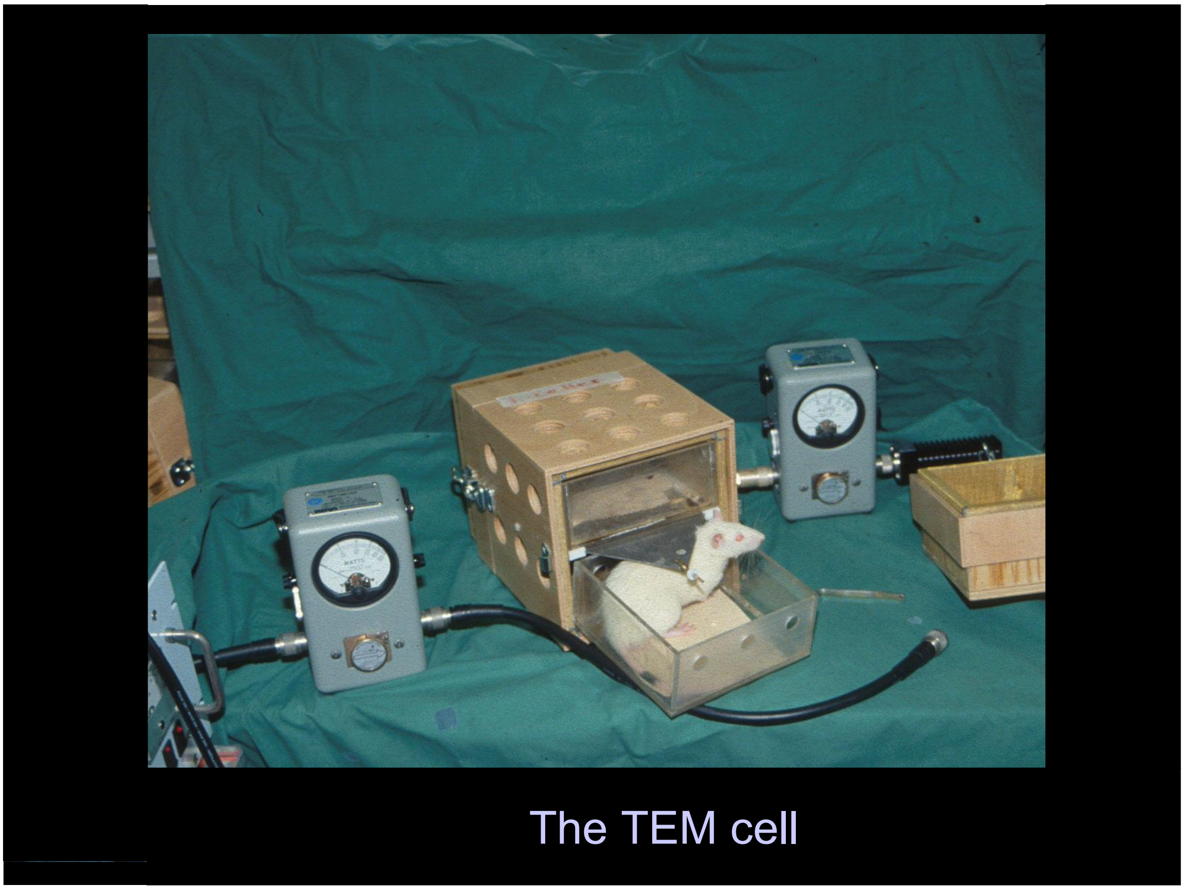
Long-term study, including studies of memory and behaviour

In a recent long-term study from our laboratory, rats were exposed to GSM radiation 2 hours weekly during 55 weeks (two different exposure groups with 0.6 mW/kg and 60 mW/kg at the initiation of the exposure period). After this protracted exposure, behaviour and memory of the exposed animals were tested. Whereas the behaviour of the animals was not affected, the GSM exposed rats had significantly impaired episodic memory as compared to the sham controls (68). After the finalization of these tests, that is 5-7 weeks after the last exposure, the animals were sacrificed by perfusion fixation. Albumin extravasation, an indicator of BBB leakage, was increased in about 1 animal in each group of low GSM exposed, high GSM exposed, sham exposed and cage control rats. About 40 % of the animals had neuronal damage. GFAP staining, as an indicator of glial reaction, revealed positive results in 31-69 % of the animals for different groups and the aggregation product lipofuscin was increased in

44-71 % of the animals for different groups. With the Gallyas staining (aiming at cytoskeletal structures), no changes were seen. When comparing the results between the different groups, it turned out that there was no statistically significant difference for any of these parameters due to GSM exposure (69). When comparing these findings to those from animals which had been exposed only once for 2 hours, it seems likely that during the 55 weeks of repeated exposure, albumin leakage at an initial stage of the experimental period might have been absorbed after some time, and that at a certain, but unknown, time point during this protracted, more than 1 year long-exposure period, some adaptation process might have been activated. However, this could not compensate for cognitive alterations, demonstrated by the episodic memory tests.

TEM-cells

In the majority of our studies, EMF exposure of the animals has been performed in transverse electromagnetic transmission line chambers (TEM-cells, see Figure 5) (53, 54, 59, 61-63, 68-71). These TEM-cells are known to generate uniform electromagnetic fields for standard measurements. Each TEM-cell has two compartments, one above and one below the center septum. Thus, two animals can be exposed at a time. The animals are un-anaesthetized during the whole exposure. Since they can move and turn in the TEM-cells as they like, the component of stress-induced immobilization (described by Stagg et al. (72)) is effectively minimized. Through our studies, we have concluded that the amount of albumin leakage is neither affected by the sex of the animals, nor their placement in the upper or lower compartments of the TEM-cells.



The TEM cell

Figure 5. TEM-cells for EMF exposure.

GSM-1800 modulated and CW microwaves in an anechoic chamber

In Lund we have also utilized an anechoic chamber for studies on microwaves from a real GSM-1800 mobile telephone, which were amplified and transferred to a dipole antenna in the anechoic chamber. The output power was varied to study the effect of various SAR values.
In a series of 65 rats exposed for 2 h with 1800-GSM at SAR: 0.027 mW/kg, and 12 rats exposed for 2 h with continuous wave, we found significantly increased albumin leakage (see figure 6) as compared to 103 control rats (p<0,03 and p<0,02, respectively). (Unpublished results).

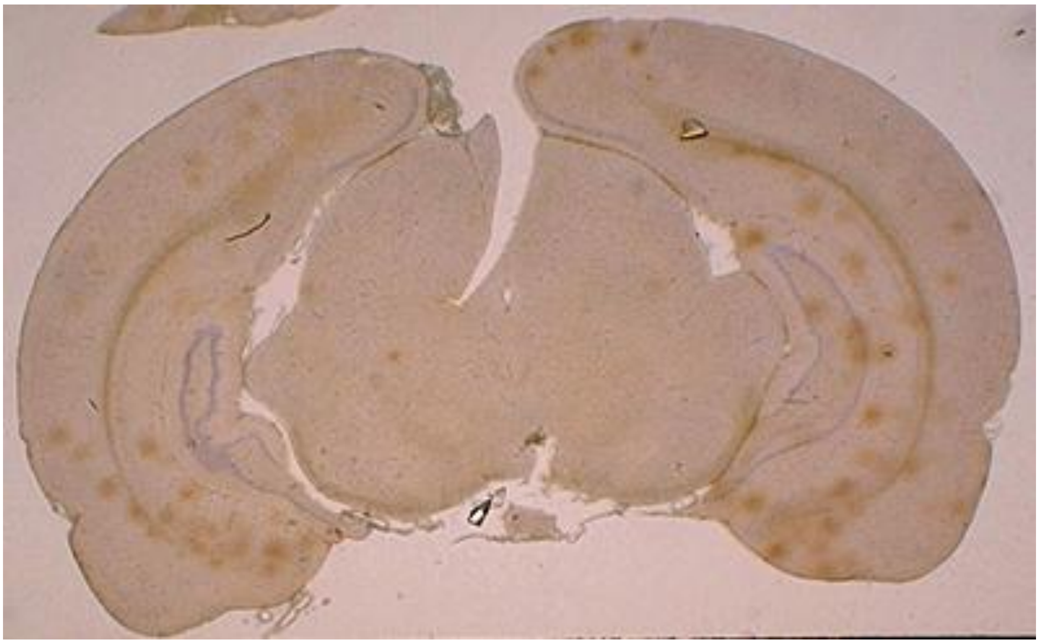


Figure 6.
Pathological leakage around vessels demonstrated by immunostaining against albumin.
Fischer 344 rat exposed for 2 h with 1800-GSM at SAR: 0.027 mW/kg

Other Studies on BBB Permeability, Focusing on the Effects of RF EMFs of the Type Emitted by Mobile Phones

With the increasing use of mobile phones, much attention has been directed towards the possible effects on BBB permeability, after exposure to the type of RF EMFs emitted by the different sorts of mobile phones.

Repetitions of our initial findings of albumin leakage have been made by Fritze et al. (73), with 900 MHz exposure of rats for 4 h at brain power densities ranging from 0.3–7.5 W/kg. Albumin extravasation into the brain tissue was seen, with significant difference between controls and rats exposed reported for 7.5 W/kg, which is a thermal level. However, Fisher exact probability test (two-tailed) performed on the reported results, reveals significant ($p < 0.01$, Fisher exact probability test) difference for the subthermal level group (SAR 0.3 W/kg plus 1.3 W/kg, compared to sham exposed and cage control animals) where in total 10 out of 20 animals showed one or more extravasations direct after exposure (Salford et al. (20)).

Another group, working in Bordeaux, and led by Prof Pierre Aubineau, has also demonstrated evidence of albumin leakage in rats exposed for 2 h to 900 MHz at non thermal SAR-values, using fluorescein-labeled proteins. The results were presented at two meetings by Töre et al. (74, 75). The findings are very similar to those of our group, described above.
At the BEMS meeting in 2002 in Quebec City in Canada, the Aubineau-Töre group presented results from exposure GSM-900 EMFs at SAR values of 0.12, 0.5, and 2.0 W/kg. Seventy Sprague-Dawley rats were included in the study. In addition to normal sham and normal GSM exposed rats, also rats subjected to chronic dura mater neurogenic inflammation, induced by bilateral sympathetic superior cervical ganglionectomy, were included. Arterial blood pressure was measured during the exposure, and Töre et al. (74, 75) concluded that the pressure variations (100–130mm Hg) were well below those limits, which are considered to be compatible with an opening of the BBB of rats. In order to induce opening of the BBB in rats, arterial blood pressure needs to reach values of 170 mmHg, according to Töre et al. (74, 75). At SAR of 2 W/kg a marked BBB permeabilization was observed, but also at the lower SAR-value of 0.5 W/kg, permeabilization, although somewhat more discrete, was present around intracranial blood vessels, both those of the meninges and of the brain parenchyma. Comparing the animals, which had been subjected to ganglionectomy, to the other animals, Töre et al. made an interesting observation: as expected, albumin extravasation was more prominent in the sympathectomised sham-exposed rats as compared to normal exposed rats. This was due to the fact that the sympathectomised rats were in a chronic inflammation-prone state with hyper-development of pro-inflammatory structures, such as the parasympathetic and sensory inputs as well as mast cells, and changes in the structure of the blood vessels. Such an inflammation-prone state has a well-known effect on the BBB leakage. However, when comparing sham-exposed sympathectomised rats to GSM-exposed sympathectomised rats, a remarkable increase in albumin leakage was present in the GSM exposed sympathectomised rats compared to the sham rats. In the GSM-exposed sympathectomised rats, both brain areas and the dura mater showed levels of albumin leakage resembling those observed in positive controls after osmotic shock. Indeed, more attention should be paid to this finding, since it implicates that the sensitivity to EMF-induced BBB permeability depends not only on power densities and exposure modulations, but also on the initial state of health of the exposed subject.

In rats, uptake of a systemically administered rhodamine-ferritin complex through the BBB also has been observed, after exposure to pulsed 2.45GHz EMFs at average power densities of

2 W/kg by Neubauer et al. (76). The authors observed that the magnitude of BBB permeability depended on power density and duration of exposure. Exposure to a lower power density (1 W/kg) and shorter duration of the exposure (15 min) did not alter the BBB permeability, as compared to higher power densities (SAR 2 W/kg) and longer duration of exposure (30–120 min). The microtubules seemed to play a vital role in the observed BBB permeability, since treatment with colchicine, which inhibits microtubular function, resulted in near-complete blockade of rhodamine-ferritin uptake. The mechanism underlying the observed leakage was presumed to be correlated to pinocytotic-like transport.

In other studies, no effect of EMF exposure has been observed on the BBB integrity. With exposure to 1,439MHz EMFs, 1 h daily during 2 or 4 weeks (average whole-body energy doses of 0.25 W/kg) no extravazation of serum albumin trough the BBB was observed in a series of 36 animals by Tsurita et al.(77). However, in this small material only 12 animals in total were EMF exposed (6 rats exposed for 2 weeks and 6 rats exposed for 4 weeks). Also, lack of interference with the BBB function of rats was found after 1,439MHz exposure for 90 min/d for 1–2 weeks at average brain power densities of either 2 or 6W/kg by Kuribayashi et al.(78). A total number of 40 animals were included in the study.

Finnie et al. (79) came to the conclusion that no increase in albumin leakage over the BBB resulted from EMF exposure in a series of 60 mice. With whole body exposure of mice to GSM-900 EMFs for 1 h at a SAR of 4 W/kg or sham exposure, no difference in albumin extravazation was observed between the different groups. Also, free-moving cage controls were included in the study, and interestingly, there was no significant difference between these non-restrained mice as compared to the sham and EMF-exposed animals. Thus, the authors concluded that there were no stress-related exposure module confinement effects on the BBB permeability.

Finnie et al. (80) continued to investigate more long-lasting exposure effects. In a series of experiments, a total of 207 mice were exposed 60 min daily, 5 days per week for 104 weeks at average whole body SARs of 0.25, 1.0, 2.0, and 4.0W/kg. This led to a minor disruption of the BBB, as seen by the use of endogenous albumin as a vascular tracer. However, it should be added that the authors performed no statistical analyses to evaluate the albumin leakage through the small vessels in the brain. In an answer to correspondence in the same journal (81), the authors presented the original data from the long-term study in one table, from which one can conclude that non-leptomeningeal albumin leaking vessels were seen in few sham-exposed animals, and in one-third of the animals in the 0.25 W/kg group and to a lesser extent in the higher SAR groups.

The fact that some research groups observe albumin leakage/transport over the BBB after EMF exposure and others do not, has led to a rather intense debate between the researchers but also in society, which is puzzled by the divergent findings. A major concentration of the involved research groups took place at Schloss Reisensburg in Germany in 2003, where the technical approaches in the studies of BBB effects were discussed. Two world-renowned researchers in the BBB field, Dr. David Begley of Kings College, London, and Prof. Olaf Poulsen of Copenhagen, Denmark, chaired the FGF/COST 281 Reisensburg, November 2–6 meeting. They made the final statement as a summary of the meeting: ‘‘It seems clear that RF fields can have some effects on tissues’’. The statement was made to a large extent on the basis of the concordant findings of the Bordeaux group, represented by Prof. Aubineau, and the Lund group, represented by Prof. Salford and Prof. Persson.

The histopathological examinations of the brains are not uncomplicated. Some laboratories that have tried to replicate our studies have not been able to demonstrate the albumin leakage. We have recently had problems with the albumin staining due to change of suppliers of avidin, biotin, serum and antibodies. The lateral hypothalamic nuclei in the immediate vicinity of the third ventricle are well known for their normally insufficient BBB. This has served as an inbuilt control of adequate albumin staining in all our experiments since 1990. In our study on combined effects of RF- and ELF-EMF, for the first time, we could not demonstrate albumin extravasation in basal hypothalamus. Not until our third attempt with new staining material, we got our positive control and could also demonstrate albumin leakage in the exposed brains (61).

The biological effects of RF exposure depend on many parameters, such as mean power level and the time variations of the power (82) and whether in vivo or in vitro experiments are performed. In the in vivo situation, different kinds of animals, and also the same kind of animals but of different breeds, might react differently. It might not necessarily be the strongest RF fields that give rise to the most obvious biological effects (54, 63). In many cases, the weak and precisely tuned EMFs have the most important biological function; two examples of this are cellular communication and protein folding. It seems quite likely that in different experimental set-ups, and in different living organisms, the signal has to be tuned to different properties in order to cause any effect. This could perhaps in some part explain why, in some cases, there are quite obvious effects of RF exposure, whereas in others, no such effects can be seen.

Other Studies on BBB permeability and neuronal damage

As has been mentioned above (p. 26) Ilhan et al. (67), in 2004 reported neuronal damage in female Wistar rats, which had been exposed to a 900 MHz electromagnetic GSM near-field signal for 1 h. a day for 7 days. They found scattered and grouped dark neurons in the cortex, hippocampus and basal ganglia, mixed in among normal neurons. A combined non-parametric test for the four groups revealed that the distributions of scores differed significantly between the control and the GSM only exposure group ($p < 0.01$).

Later, Masuda et al. (83) tried to replicate the findings by our group of albumin extravasation and dark neurons. F344 rats (n=64) were exposed to 915 MHz signals for 2 hours (SAR of 0, 0.02, 0.2 and 2 W/kg), and albumin extravasation and dark neurons were investigated 14 and 50 days after the exposure. No albumin extravasation was seen, neither in control or exposed rats, and no difference in the occurrence of dark neurons could be found due to EMF exposure. An interesting difference as compared to the studies by Salford et al. mentioned above, was that animals, after perfusion fixation, were left in a 4ºC storage for 18 hours before the brains were removed. The question is whether this might have led to dilution of the very sensitive albumin extravasation, which is often more pronounced in the circumventrical organs as compared to the brain extravasates (personal communications with our neuropathologist Arne Brun). This might explain the fact, that no albumin extravasation could be seen in neither the cage control animals, the shams or the GSM exposed animals.

Another study by Mason and his group at Brooks Airforce Resarch Laboratory, San Antonio, also tried to confirm our findings of albumin extravastion by using the same type of TEM-cells for EMF Exposure (84), although the exposure parameters where somewhat different with only 30-min exposure, including only male rats of the Fischer 344 CD-VAF strain and utilizing only the upper compartment of the TEM cells. Exposure was at whole-body SAR values of 0.002 to 20 W/kg. Regarding extracellular albumin accumulation, the results were not formally analyzed, as motivated by too low scores of albumin. Regarding intracellular albumin uptake, no significant difference between the different groups was reported. However, as presented in the paper by McQuade et al.(84), at the lowest SAR of 1.8 mW/kg at 16 Hz, of 33 exposed rats, 11 had 2 or 3 positivities (33% of the animals) and 22 had none or 1 positivity. In the sham animals, 18% were positive and among the cage controls only 12%. These results are reminiscent of prior work by the Lund group reporting that 17% of the sham animals had some albumin leakage, while only at the most 50% of the identical and equally handled, but RF exposed animals displayed albumin extravasation (60).

In a third study aiming to replicate the Lund findings of dark neurons, a group in Bordeaux (85) exposed 14 weeks old Fischer 344 rats (which, however, were restrained in a rocket-type exposure setup), to the GSM-900 signal for 2 h at various brain-averaged SARs (0, 0.14 and 2.0 W/kg). Eight rats were included in each of these groups.
Albumin leakage and neuronal degeneration was evaluated 14 and 50 days after exposure.
It was reported that no statistically significant albumin leakage was observed and that neuronal degeneration assessed using cresyl-violet or the more specific marker Fluoro-Jade B, was not significantly different among the tested groups. Here we want to point out that the Bordeaux group makes a major deviation from the way we have evaluated the occurrence of dark neurons in the tissue slices. While we counted the overall number of dark neurons, de Gannes et al. (85) chose to subdivide the slices into 12 different small regions, which were compared individually to each other (fig 3 in the publication). This gave the effect that a clear overall difference in number of observed dark neurons between animals 50 days after exposure to 2 W/kg for two hours versus sham exposed, disappeared in the statistics. On the contrary, if all the numerical values for the bars representing the scored dark neurons observed in each brain zone and region 50 days after exposure to 2 W/kg are compared to all those of the sham animals, a highly significant difference (Kruskall-Wallis) between animals exposed to 2 W/kg and sham is demonstrated (Mann-Whitney) $p = 0.003$! This is in concordance with the Lund experience!

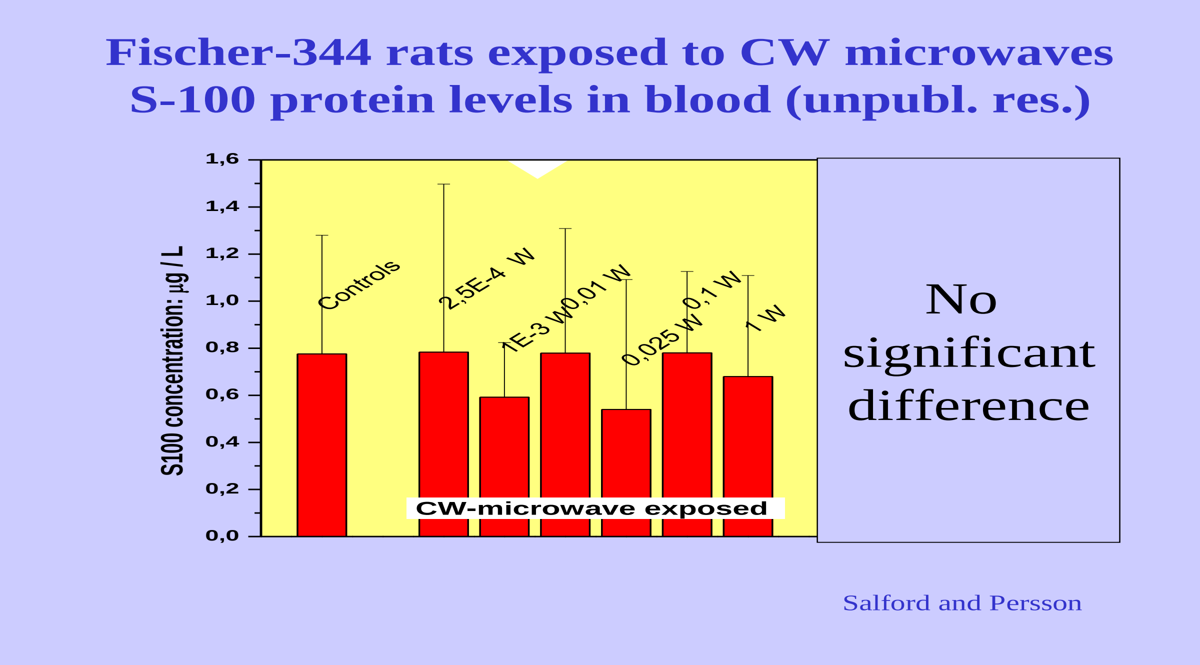
Indirect studies and studies on the blood cerebrospinal fluid barrier

The integrity of the BBB has also been investigated indirectly. Cosquer et al. (86) treated rats with the muscarinic antagonist scopolamine methylbromide, which is known to induce memory impairments, followed by EMF exposure at 2.45GHz for 45 min at average whole body SARs of 2W/kg. Opening of the BBB after EMF exposure was hypothesised to affect the performance in a radial arm maze. However, no such alterations were observed and the authors concluded that no BBB opening seemed to have occurred. In agreement with this, no albumin extravasation was noticed.

Ushiyama et al. (87) investigated the effects on the blood cerebrospinal fluid barrier after RF-EMF exposure. With a microperfusion method, cerebrospinal fluid from rat brain was collected in vivo. Fluorescent intensity of FITC-albumin in perfusate was measured. Rats exposed to 1.5GHz RFs during 30 min at SAR-values of 0.5, 2.0, 9.5W/kg for adult rats and 0.6, 2.2, 10.4W/kg for juvenile rats, respectively, were compared to sham-exposed controls. Under these conditions, no increase in FITC-albumin was seen in the cerebrospinal fluid of exposed rats as compared to sham exposed controls. It was concluded that no effect on the function of the blood cerebrospinal fluid barrier was seen.

In a recent study, the permeability of the human BBB after mobile phone exposure was assessed measuring blood levels of S100B and transthyretin in human volunteers by Söderqvist et al. (88). S100B is a calcium-binding protein, and it has been shown to be increased in serum after damage to the BBB. Transthyretin, also known as pre-albumin, is synthesised both in the liver and the choroid plexus. 30 min of GSM-900-like exposure at SAR-values of 1 W/kg was used. No difference was seen regarding S100, but transthyretin was increased 60 min after the termination of exposure as compared to the control situation. The concentrations of S100B and transthyretin were also analysed 30 min prior to provocation and after 30 min rest, showing a decrease after 30 min rest, which was suggested, might be due to less stress after the 30 min rest. Thus, it is interesting that despite this decline, which might be due to relaxation, still an increase in thransthyretin could be measured 30 min after exposure. It was also put forward, that it could not be excluded that the thransthyretin rise might be a compensation to the previous decrease, and that new studies including more participants and also a sham group would be needed.

We have in the past investigated whether MW exposure, CW and at different SAR levels might enhance S-100 protein levels in the blood of a large proportion of our rats. We could conclude that no significant differences were seen (see Figure 7 below) (to be published).



Fischer-344 rats exposed to CW microwaves
S-100 protein levels in blood (unpubl. res.)

No significant difference

Salford and Persson

Figure 7. S-100 in the blood of rats after EMF exposure (to be published in Acta Scientiarum Lundensia).

In another study, by Sirav and Seyhan (89), exposure to CW EMFs at 900 and 1,800 MHz for 20 min, increased the BBB permeability of male but not female rats. Evans blue dye, which binds to serum albumin after injection, was used to quantitatively measure BBB permeability. A strength of this study, was the ability to objectively quantify the Evans blue uptake in the brain. The finding that only male, and not female rats, are affected, is however not fully addressed.

In Vitro Models

In recent years, there has been an increasing use of in vitro models in the search for BBB effects of EMF exposure. In vitro models of the BBB have been studied, as by Schirmacher et al. (90), with co-cultures consisting of rat astrocytes and porcine brain capillary cells. Exposure to GSM-1800 for 4 d with average SAR of 0.3 W/kg increased the permeability of 14C-sucrose significantly compared to unexposed samples in the studied BBB model. These findings were not repeated in experiments performed later by the same group, after modifications of their in vitro BBB model (91). The modified BBB model had a higher general tightness. It was speculated that at a higher original BBB permeability, which was present in the first study by Schirmacher et al. (90), the cultures were more susceptible to the RF EMFs. Using porcine brain microvascular-endothelial cell cultures as an in vitro model of the BBB, no effects on barrier-tightness, transport behavior, and integrity of tight junction proteins were observed-after exposure to UMTS EMFs at 1.966 GHz for 1–3 d at different field strengths at 3.4–34 V/m, generating a maximum SAR of 1.8 W/kg (92).

In the search after the mechanism underlying non thermal EMF effects, Leszczynski et al. (93) observed human endothelial cells, with the interesting finding that GSM-900 exposure for 1 h with SAR-values of 2 W/kg resulted in changes in the phosphorylation status of many proteins. Among the affected pathways, the hsp27/p38MAPK stress response pathway was found, with a transient phosphorylation of hsp27 as a result of the mobile phone exposure. This generated the hypothesis that the mobile-phone induced hsp27-activation might stabilize stress fibers and in this way cause an increase in the BBB permeability. Furthermore, it was also suggested that several brain-damaging factors might all contribute to the mobile phone-induced effects observed in the brain and other structures as well.

Further perspectives of the importance of the BBB including the human situation

BBB in the Context of Alzheimer's Disease and the findings by the Zlokovic Group

The BBB, as mentioned previously, is of essential role for maintaining an accurate brain function. As described by Zlokovic (94), in a review regarding BBB in correlation to neurodegenerative disorders, BBB breakdown can be due to tight junction disruption, alterations of angiogenesis or vessel regression, hypoperfusion, inflammatory response and alterations of the transport of molecules across the BBB (94). Further, as Zlokovic hypothesises, this might contribute to neurodegenerative disorders, such as Alzheimer's disease (AD), Parkinson's disease, multiple sclerosis and amyotrophic lateral sclerosis.

In the review by Zlokovic (94), a neurovascular disease pathway is presented, regarding possible genesis of AD, where it is suggested that changes in vascular genes and receptors in brain capillaries and small arteries might disrupt BBB functions, leading to an accumulation of amyloid beta (Aβ), a neuroinflammatory response and BBB breakdown and further on accumulation of Aβ, loss of the BBB to clear Aβ (due to affected synaptic transmission, neuronal injury and recruitment of microglia) and secretion of proinflammatory cytokines. Ultimately, this is suggested to lead to disappearance of the capillary unit, increasing Aβ deposits and synaptic and neuronal loss (94).

This observation might explain how vascular disease contributes to Alzheimer's disease (AD) risk; the heterogeneity of AD; and supports the idea that exclusively focusing on amyloid is likely to be disappointing.

Neuronal injury resulting from vascular defects that are not related to amyloid-beta but is related to damage results from a breakdown of the blood-brain barrier and a reduction in blood flow (94). Although Amyloid beta definitely has an important role in Alzheimer's disease it's very important to investigate other leads, perhaps where amyloid-beta isn't as centrally involved.

Human apolipoprotein E has three isoforms: APOE2, APOE3 and APOE4. APOE4 is a major genetic risk factor for Alzheimer's disease and is associated with Down's syndrome dementia and poor neurological outcome after traumatic brain injury and haemorrhage. Neurovascular dysfunction is present in normal APOE4 carriers and individuals with APOE4-associated disorders. In mice, lack of APOE leads to blood-brain barrier (BBB) breakdown, whereas APOE4 increases BBB susceptibility to injury. How APOE genotype affects brain microcirculation remains elusive. Using different APOE transgenic mice, including mice with ablation and/or inhibition of cyclophilin A (CypA), it has been shown show that expression of APOE4 and lack of murine APOE, but not APOE2 and APOE3, leads to BBB breakdown by activating a proinflammatory CypA-nuclear factor-kappa B-matrix-metalloproteinase-9 pathway in pericytes. These findings suggest that CypA is a key target for treating APOE4-mediated neurovascular injury and the resulting neuronal dysfunction and degeneration. The data reviewed above support an essential role of neurovascular and BBB mechanisms in contributing to both, onset and progression of AD (95, 96).

BBB in the context of Alzheimer's Disease – Importance of EMF Exposure

In this context, the findings of Arendash et al., that long-term EMF reduced brain Aβ deposition through Aβ anti-aggregation actions in AD mice, are highly interesting (97). It was also found, by Mori and Arendash et al., that long-term exposure to high frequency EMF treatment prevented cognitive impairment in AD transgenic (Tg) mice and improved memory in normal mice and that an increase in neuronal activity could be observed in the EMF exposed groups (98). Furthermore, it was found by the group that EMF treatment enhances brain mitochondrial functions in AD Tg as well as normal mice and that no increase in brain temperature could be found in connection to the EMF exposure (99). An interesting aspect in this context, is the role of mitochondria for many cellular functions, including reactive oxygen species generation, apoptosis, and $Ca2^+$ homeostasis as was mentioned by Dragicevic et al. and reviewed by Nicholls (99, 100).

In the first mentioned study by Arendash et al. (97), mice were EMF exposed with start at young age or at adult age. In the young-age group, 24 mice were divided into 4 subgroups: n=6 were Tg controls, n=6 were Tg animals treated with EMF, n=6 were non-transgenic (NT) controls and n=6 were NT animals treated with EMF. 2.5, 4-5 and 6-7 months after daily GSM-900 EMF exposure (two 1-hour sessions daily, at SAR 0.25 W/kg), the animals were evaluated by cognitive tests. At the end of the study, Aβ in the brains was evaluated by immunohistochemistry. No effect on cognitive functions was observed after 2 months of exposure. However, for the Tg+EMF mice with start of EMF exposure at young age, the cognitive function was maintained after 6-7 months of exposure, while it deteriorated in the Tg group. In a final task for NT mice after 7 months of EMF, the EMF actually improved the mnemonic function. In the adult-age group, Tg animals had impaired cognitive functions at the age of 4 months. 28 Tg and NT mice were included. After long-term EMF exposure (2, 5 and 8 months) the memory was tested. While 2 months of EMF exposure had no effect, 5 months of exposure had positive effects only on NT mice, and 8 months of exposure had beneficial effects for the Tg mice, with better results in the Tg+EMF group as compared to the Tg controls. Also the NT+EMF mice had an improved function as compared to NT controls after 8 months. Staining for Aβ revealed lower values on both hippocampus and the entorhinal cortex in the Tg+EMF group as compared to the Tg control group. Hippocampal tissue from Tg mice were then exposed to EMF for 4 days, after which it was shown that the Aβ amount had decreased as compared to non-exposed control tissue. It was also reported that a ~~t~~1° temperature increase was observed in EMF exposed animals during exposure, but not in between exposure sessions (97).

In the study by Mori and Arendash (98), n=6 mice were Tg controls, carrying the mutant APPK670N , n=10 mice were Tg treated with EMF, n=4 mice were NT controls and n=5 mice were NT treated with EMF. EMF exposed animals were placed in a Faraday cage, receiving two 2-hour periods of EMF treatment at GSM-900 frequencies, pulse modulated at SAR 0.25-1.05 W/kg. The neuronal expression of c-Fos was taken as an indicator of neuronal activity. With immunohistochemistry, it was found that c-Fos was increased in both the NT+EMF group, as well as in the Tg+EMF group in the entorhinal cortex. However, only this one brain region was analyzed, since c-Fos expression was too low in other regions, which the authors hypothesised might be due to that c-Fos in an early response gene, and that at a certain time after stimulation, when the animals were sacrificed, the expression had already declined in other regions, such as hippocampus. In a cognitive test (Y-maze), it was found that EMF improved the performance in both NT and Tg group as compared to untreated controls. It should also be noted, that despite the very interesting findings, the number of included animals is quite small (98).

EMF and ^{18}FDG Uptake – Recent Studies

The question whether EMF exposure from mobile phones has neuronal effects in the human situation was recently addressed by an American research group led by Volkow et al., conducting a PET study on ^{18}F-fluorodeoxyglucose (^{18}FDG) uptake (101). Though PET-studies on humans in correlation to EMF exposure have also been previously made, the purpose of this study was to extend the study material and use the more direct measure of brain glucose metabolism by the uptake of ^{18}FDG instead of the previously used CBF (cerebral blood flow) measure, which might be a more indirect sign of neuronal activity and also reflect short-term alterations (60s) as compared to the more long-lasting ones observed with ^{18}FDG (suggested to be in the range of 30 min). ^{18}FDG is actively transported across the BBB into the cells, where it is phosphorylated, and is, among others, used as a prognostic value for following low-grade brain tumours, where an increased uptake in previously low-grade tumours is an indicator of anaplastic transformation (for review into the topic of ^{18}FDG and brain tumours (102).

(space)

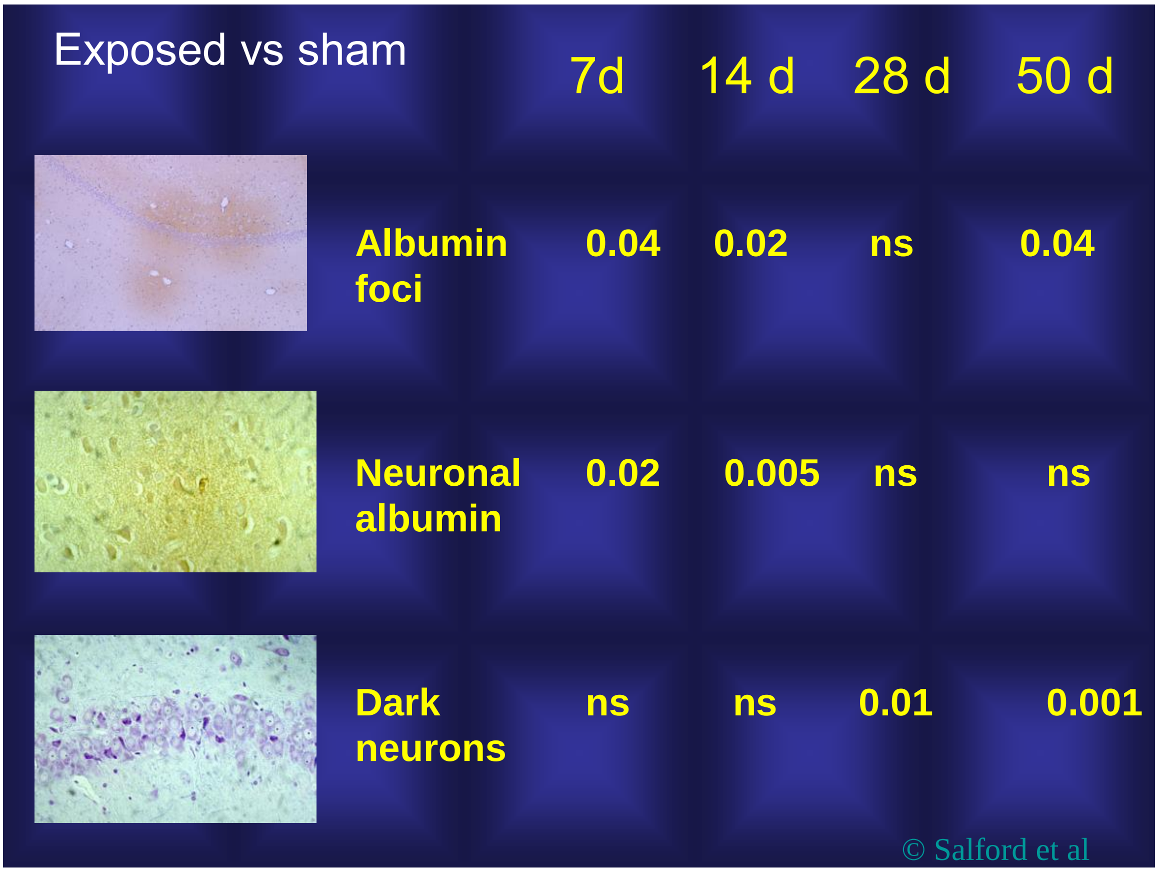
In the study by Volkow et al. (101), in total, 47 persons were involved, and effects upon brain glucose metabolism of EMF exposure were evaluated using PET with injection of ^{18}FDG. PET scans were performed both with and without EMF exposure (50 min of GSM-900 with maximum SAR of 0.901 W/kg), and the participants were blinded to the exposure situation. Whereas whole-brain metabolism was not affected, there were regional differences, in the right orbitofrontal cortex and the lower part of the right superior temporal gyrus (that is, the same side as the mobile phone was placed at) with increased metabolism in the exposure situation of about 7% as compared to control. There was a positive correlation between the strength of the E-field from the phones and the brain activation. Interestingly, it was hypothesized that RF-EMF exposure might increase the excitability of brain neurons.

Following the study by Volkow et al. (101), Kwon et al. (103) also investigated effects of GSM-900 exposure upon brain ^{18}FDG uptake. Thirteen persons were exposed to GSM-900 for 30 minutes to the right side of the head, and all subjects were also sham-exposed, and blinded to the exposure situation (SAR-values of maximum 0.74 W/kg in the head and 0.23 W/kg in the brain tissue). Contrary to the findings of Volkow et al. (101), the study by Kwon et al. (103) demonstrated a decrease in brain ^{18}FDG uptake after GSM-900 exposure, with decreased uptake values in the temporoparietal junction. A volume-of-interest analysis focused upon the right temporal lobe, showed a decreased ^{18}FDG uptake in the anterior inferior temporal cortex. No effects on task performance were found, and no correlation between temperature or ^{18}FDG uptake (a temperature increase of <0.21°C was found on the skin on the exposed side of the head) (103).

In the animal situation, Frilot et al. investigated the effect of ELF magnetic field exposure (2.5 G at 60 Hz) upon ^{18}FDG uptake in rats, comparing uptake with and without EMF exposure. An increased glucose uptake was found in the hindbrain when the field was orthogonally to the sagittal plane, but not when the angle varied randomly between the field and sagittal plane. These effects were hypothesized to be coupled to induction of electric field on the gate of ion channels (104).

Possible connection between BBB leakage and nerve cell injury

It has been suggested that BBB leakage is the major reason for nerve cell injury, such as that seen in dark neurons in stroke-prone spontaneously hypertensive rats (105). Much speaks in favour of this possibility. The parallel findings in the Lund material of neuronal uptake of albumin and dark neurons may support the hypothesis that albumin leakage into the brain is the cause for the neuronal damage observed after 28 and 50 d. It should, however, be pointed out that the connection is not yet proven (Figure 8).



Exposed vs sham	7d	14 d	28 d	50 d
Albumin foci	0.04	0.02	ns	0.04
Neuronal albumin	0.02	0.005	ns	ns
Dark neurons	ns	ns	0.01	0.001

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Figure 8. Results from the Lund group (61-63)

Also, other unwanted and toxic molecules in the blood may leak into the brain tissue in parallel with the albumin, and concentrate in and damage the neurons and glial cells of the brain. In favour of a causal connection between albumin and neuronal damage is a series of experiments performed in rats by another group at Lund University; albumin leaks into the brain and neuronal degeneration is seen in areas with BBB disruption in several circumstances: after intracarotid infusion of hyperosmolar solutions in rats (106) in the stroke prone hypertensive rat (105); and in acute hypertension by aortic compression in rats (22). Furthermore, it has been shown in other laboratories that epileptic seizures cause extravasation of plasma into brain parenchyma (21), and in the clinical situation the cerebellar Purkinje cells are heavily exposed to plasma constituents and degenerate in epileptic patients . There are indications that an already disrupted BBB is more sensitive to the RF fields than an intact BBB (74, 91). It has been stated by other researchers that albumin is the most likely neurotoxin in serum (64). It has been demonstrated that injection of albumin into the brain parenchyma of rats gives rise to neuronal damage. When 25 micro-litres of rat albumin is infused into rat neostriatum, 10 and 30, but not 3 mg/ ml albumin causes neuronal cell death and axonal severe damage (65). It also causes leakage of endogenous albumin in and around the area of neuronal damage. However, it is still unclear whether the albumin leakage demonstrated in our experiments locally reaches such concentrations.

Possible mechanisms

Microarray analysis of the expression of all the rats' genes in cortex and hippocampus, after exposure to GSM RFs or sham exposure for 6 h, has shown interesting differences between exposed animals and controls as described by Nittby et al. (107). Genes of interest for membrane transport show highly significant differences. This may be of importance in conjunction with our earlier findings of albumin leakage into neurons around capillaries in exposed animals. It can be noted here that among the significantly altered genes from these evaluations, two variants of the gene RGS4 are up-regulated in hippocampal tissue from exposed rats as compared to the sham-exposed rats (unpublished results). RGS is a regulator of G protein signalling, and it has been proposed that RGS4 might regulate BBB permeability in mammals, in a way corresponding to the role of its Loco homolog G protein coupled receptor (GPCR) in developing and maintaining the BBB permeability of Drosophila (7).

It has also been suggested in other connections that manifestations of BBB disruption might also be mediated by the formation of free radicals, such as O_2^-, H_2O_2, and hydroxyl radical, which are supposed to oxidize cell membrane lipids by virtue of the high concentration of polyunsaturated fatty acids in these membrane constituents (108). As an example of this, it was reported by Chan et al.(109), that treatment of the brain of rats with a free-radical generating system resulted in lipid-peroxidation, and an increased permeation of Evans blue due to barrier breakdown.

Recently, a detailed molecular mechanism, by means of which mobile phone radiation might exert its effects, has been proposed (110). By using Rat1 and HeLa cells, it was shown that EMF exposure resulted in rapid activation of ERK/ MAPKs (mitogen-activated protein kinase). The activation of these ERKs was mediated by reactive oxygen species (ROS), resulting in a signalling cascade ultimately affecting transcription, by the central key role of ERKs in signalling pathways.

In the continued search for the mechanisms behind EMF mediated effects, their interaction with calcium-45 transport in bio-membranes has been studied (111) and Ca2^{+}-efflux over plasma membranes has been observed in plasma vesicles from spinach exposed to ELF magnetic fields (112). With this model, quantum mechanical theoretical models for the interaction between magnetic fields and biological systems are tested. The model proposed by Blanchard and Blackman (113), in which it is assumed that biologically active ions can be bound to a channel protein and in this way alter the opening state of that channel, could in this way be quantitatively confirmed. Thus, the membrane is one site of interaction between the magnetic fields and the cell, and more specifically, the Ca2^{+}-channels, are one of the targets. More recently, new models for the interaction between magnetic fields and hydrogen nuclei also have been proposed.

EMF-induced Ca2^{+}-efflux over plasma membranes, understandably, can have many different effects on the target cells. Some agents that increase the BBB permeability act through a contractile mechanism that widens the intercellular junctions of the capillary endothelium. An increase of free Ca2^{+} should mediate these changes, thereby resulting in measurable alterations of intracellular Ca2^{+}-levels in brain capillary cells after exposure to BBB-disrupting agents (108).

Another hypothesis is that EMF-induced intracellular Ca2^{+}-alterations might affect Ets genes, which are transcription factors expressed in different tissues (114). In this context, we could add that in our gene expression material from GSM-exposed rats vs., sham-exposed rats, one Ets variant gene is actually significantly up-regulated in hippocampus and one Ets1 gene is significantly up-regulated in cortex of the exposed animals.

EMF induced BBB permeability – with the aim of medical use

In the attempt to further try to understand the underlying mechanisms of the RF effects, we recently undertook a study upon snail nociception, with 1-hour GSM-1800 exposure of the land snail *H. pomatia*. This revealed, that the exposure induced analgesia in the snail model, with a significantly increased latency of reaction when placed on a hot plate, as compared to when only sham exposed. The vast knowledge about the physiology of the snail, its neurotransmission systems and it simplicity as compared the mammals may provide a tool for successful continued search for the mechanisms behind the effects of the GSM EMF upon biology (115).

In a recent study by Kuo et al (116), it was described how EMFs might be utilized to facilitate transport across the BBB. In an *in vitro* model, human micro-vascular endothelial cells were co-cultured with human astrocytes. Effects of EMF upon P-glycoprotein (P-gp) and multi-drug resistance -associated proteins (MRP) were tested in connection to treatment with anti-retroviral drugs, where the MRPs and P-gp are known to play an important role in multidrug resistance, which is encountered in carcinomas and therapies for acquired immune-deficiency (Kuo et al. 2012). With increasing EMF frequencies up to 900 MHz (both 715MHz and 900 MHz), the endocytotic uptake of calcein was increased (5mW, square wave with amplitude modulation at 20 MHz for 4 hours). Treatment with EMF could also inhibit expression of MRP and P-gp after treatment with anti-retroviral drugs, indicating that it might be useful in order to deliver antiretroviral proteins into the brain, by decreasing the efflux of the drugs due to the MRPs and P-gl.

Kuo et al. (117) also showed that EMF exposure (915 MHz EMFs at 5 mW with 20 MHz amplitude modulation for 4 hours) in combination with cationic solid lipid nanoparticles (CSLNs) could increase the transport of the antiretroviral drug Saquinavir 22-fold across human brain-microvascular endothelial cells (as compared to a 17-fold increase when only CSLNs were used).

Conclusions

In this review, we have reported the results of our group's research during the last 24 years, and the results of similar, but seldom identical, experiments of several other groups around the world. When summing up what we have described here, we are convinced that RF electromagnetic fields have effects upon biology, and we believe that it is more probable than unlikely, that non-thermal electromagnetic fields from mobile phones and base stations do have effects also upon the human brain. However, in this context, it is also important to point out, that the studies from our laboratory, as well as most studies presented above and available in literature, have been performed using animals and not humans. Thus no definitive conclusions can be drawn regarding effects of mobile phone use upon the human BBB.

However, studies in humans utilizing radiopharmaceuticals have been performed by Volkow et al. (101) upon brain glucose metabolism, and as was described by Saha et al. (118) already in 1994, studies with PET or SPECT and radiopharmaceuticals are used in brain imaging.

Further, a tool to directly study the human BBB has recently been described (119). It is based upon a non-radioactive methodology for *in vivo* non-invasive, real-time imaging of BBB permeability for conventional drugs, using nitroxyl radicals as spin-labels and MRI. In this connection, it should be mentioned though, that MRI has the drawback of possibly itself influence upon the results.

Based upon what has been presented here, we feel that the WHO IARC classification of RFR at the level 2B is adequate at present.

The question whether existing FCC/IEE and/or ICNIRP public safety limits and reference levels are adequate to protect the public is not easily answered. The reported studies on EMF induced BBB disruption have shown partially contradictory results from different laboratories. However, the fact that an abundance of studies do show effects is an important warning. This is true even if it can be summarized that the effects most often are weak and are seen in about 40% of the exposed animals.

However, we have stressed the following opinion in several publications during the past years: - "*The intense use of mobile phones, not least by youngsters, is a serious memento. A neuronal damage may not have immediately demonstrable consequences, even if repeated. It may, however, in the long run, result in reduced brain reserve capacity that might be unveiled by other later neuronal disease or even the wear and tear of ageing. We can not exclude that after some decades of (often), daily use, a whole generation of users, may suffer negative effects such as autoimmune and neuro-degenerative diseases maybe already in their middle age*".

One remarkable observation, which we have made in our studies throughout the years, is that exposure with whole-body average power densities below 10 mW/kg gives rise to a more pronounced albumin leakage than higher power densities, all at non-thermal levels. These very low SAR-values, such as 1 mW/kg, exist at a distance of more than one meter away from the mobile phone antenna and at a distance of about 150–200 m from a base station. Further, when a mobile phone operating at 915 MHz (and its antenna) is held 1.4 cm from the human head, the very low SAR levels of 10 mW/kg exist in deep-lying parts of the human brain such as the basal ganglia, and the power density of 1 mW/kg and less is absorbed in thalamus bilaterally.

With this information as a background, it is difficult to recommend safety limits as the function of existing mobile systems might not allow for limits that produce SAR levels below 1 or 0,1 mW/kg in the human brain, which are reported to cause a pathological leakage of the BBB and to neuronal damage.

Demonstrated effects on the BBB, as well as a series of other effects upon biology (120) have given rise to scientific concern and to public anxiety. It is up to the society and our politicians and also the providers of the radiofrequency-emitting technologies to support continued research in order to understand the nature of the effects, thereby neutralizing or at least reducing them. Also, it should be kept in mind that proven effects on biology also means that positive potentials might be revealed. This might be useful in medical applications, for example a controlled opening of the BBB would enable previously excluded pharmaceuticals to reach their targets within the brain tissue.

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SECTION 11- part 1

Evidence For Brain Tumors And Acoustic Neuromas

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Table 1 **Summary of 20 studies on the use of cellular telephones and brain tumor/acoustic neuroma risk**

I. Introduction

During the recent decade potential health risks from microwave exposure during use of wireless phones has been discussed both in scientific settings but also by the layman. Especially the use of mobile phones has been of concern, to less extent use of cordless desktop phones (digital enhanced cordless telephone; DECT). The Nordic countries were among the first in the world to widely adopt use of such devices, probably due to the mobile phone companies like Ericsson in Sweden and Nokia in Finland.

These countries may be taken as models for the introduction of this new technology on the market. Thus, the analogue mobile phone system (Nordic Mobile Telephony, NMT) using 450 MHz started to operate in Sweden in 1981. First, it was used in cars with external antenna but from 1984 mobile (portable!) phones existed. This system is still used in Sweden but only to a minor extent. The 900 MHz NMT system operated in Sweden between 1986-2000. The GSM phone (Global System for Mobile communication) started in 1991 and is the most used phone type today, although the 3G phone (third generation mobile phone, UMTS) is increasingly used now.

The risk of brain tumors has been of special concern since the brain is the organ mainly exposed during such phone calls. Most studies on this topic have been of the case-control design and no results exist from prospective cohort studies. However, the results have been hampered by too short tumor-induction period in most studies or with limited number of long-term users, i.e. $\geq$ 10 years latency time. As to carcinogenesis short latency period is of limited value to predict long-term health risks. Usually a latency period of at least 10 years is needed for more firm conclusions. It should noted that for several carcinogens longer latency periods are often required, such as smoking and lung cancer, asbestos and lung cancer, dioxins and certain cancer types etc.

By now a number of studies exist that give results for brain tumour risk and use of mobile phones for subjects with latency period ≥ 10 years. Most of these results are based on low numbers but nevertheless may together give a pattern of increased risk. In this review we discuss all studies on this topic that have been published so far. Moreover, we present a meta-analysis of results from studies with at least 10 years latency period. Only the Hardell group in Sweden has published results also for use of cordless phones. Recently the same group published an overview of long-term use of cellular phones and the risk for brain tumors, especially with use for 10 years or more (Hardell et al 2007). In the following a brief summary is given of these results with the addition of two more study published after that review (Klaeboe et al 2007, Schlehofer et al 2007). For further details see Hardell et al (2007).

II. Materials and Methods

The Pub Med database (**www.ncbi.nlm.nih.gov**) was used for an up-dated search of published studies in this area using mobile/cellular/cordless telephone and brain tumour/neoplasm/acoustic neuroma/meningioma/glioma as searching terms. Personal knowledge of published studies was also used in order to get as comprehensive review as possible. Regarding several publication of the same study the most recent one with relevant data was used. We identified 20 studies to be included. Two were cohort studies (one study analysed twice) and 18 were case-control studies. No mortality studies were included. Three studies came from USA, four from Denmark, one from Finland, five from Sweden, two from Germany, one from the UK, one from Japan, one from Norway and two from study groups partly overlapping previously mentioned studies.

III. Results

A. The first Swedish studies

The first study by Hardell et al (1999, 2001) included cases and controls collected during 1994-96 in Sweden. Only living cases were included. Two controls were selected to each case from the Population Registry. The questionnaire was answered by 217 (93 %) cases and 439 (94 %) controls. Overall no association between mobile phone use and brain tumours was found, but when analysing ipsilateral phone use a somewhat increased risk was seen especially for tumours in the temporal, occipital or temporoparietal lobe yielding odds ratio (OR) = 2.4, 95 % confidence interval (CI) = 0.97-6.1 (Hardell et al 2001).

Hardell et al (2006a) made a pooled analysis for benign brain tumours from their two case-control studies. Cases were reported from Cancer Registries and controls were population based. The questionnaire was answered by 1,254 (88 %) cases and 2,162 (89 %) controls. Also use of cordless desktop phones was assessed. Use of cellular phones gave for acoustic neuroma OR = 1.7, 95 % CI 1.2-2.3 increasing to OR = 2.9, 95 % CI = 1.6-5.5 with > 10 year latency period. The corresponding results for cordless phones were OR = 1.5, 95 % CI = 1.04-2.0, and OR = 1.0, 95 % CI 0.3-2.9, respectively. Regarding meningioma cellular phones gave OR = 1.1, 95 % CI = 0.9-1.3, and cordless OR = 1.1, 95 % CI = 0.9-1.4. Using > 10 year latency period ORs increased, for cellular telephones OR = 1.5, 95 % CI = 0.98-2.4, and for cordless phones OR = 1.6, 95 % CI = 0.9-2.8.

The pooled analyses of the two case control studies of malignant brain tumours by Hardell et al (2006b) included 905 (90%) cases and the same control group as for benign tumours was used, 2,162 (89 %) subjects. Overall for low-grade astrocytoma cellular phones gave OR= 1.4, 95 % CI = 0.9-2.3 and cordless phones OR = 1.4, 95 % CI = 0.9-3.4. The corresponding results for high-grade astrocytoma were OR = 1.4, 95 % CI = 1.1-1.8, and OR = 1.5, 95 % CI = 1.1-1.9, respectively. Using > 10 year latency period gave for low-grade astrocytoma and use of cellular phones OR = 1.5, 95 % CI = 0.6-3.8 (ipsilateral OR = 1.2, 95 % CI = 0.5-5.8), and for cordless phones OR = 1.6, 95 % CI = 0.5-4.6 (ipsilateral OR = 3.2, 95 % CI = 0.6-16). For high-grade astrocytoma in the same latency period cellular phones gave OR = 3.1, 95 % CI = 2.0-4.6 (ipsilateral OR = 5.4, 95 % CI = 3.0-9.6), and cordless phones OR = 2.2, 95 % CI = 1.3-3.9 (ipsilateral OR = 4.7, 95 % CI = 1.8-13).

B. Studies from USA

Muscat et al (2000) studied patients with malignant brain tumours from five different hospitals in USA. Controls were hospital patients. Data from 469 (82 %) cases and 422 (90 %) controls were available. Overall no association was found, OR for handheld cellular phones was 0.9, 95 % CI = 0.6-1.2, but the mean duration of use was short, only 2.8 years for cases and 2.7 years for controls. For neuroepithelioma OR = 2.1, 95 % CI = 0.9-4.7, was reported. The study is inconclusive since no data were available on long-term users (≥ 10 years latency period). Some support of an association was obtained since of 41 evaluable tumours, 26 occurred at the side of the head mostly used during calls and 15 on the contralateral side.

Also the study by Inskip et al (2001) from USA had few long-term users of mobile phones, only 11 cases with glioma, 6 with meningioma and 5 with acoustic neuroma with ≥ 5 years regular use. No subjects had ≥ 10 years use. The study comprised 489 (92 %) hospital cases with malignant brain tumours, 197 with meningioma and 96 with acoustic neuroma, and 799 (86 %) hospital-based controls. Overall no significant associations were found. Regarding different types of glioma OR = 1.8, 95 % CI = 0.7-5.1 was found for anaplastic astrocytoma. Duration of use ≥ 5 years gave for acoustic neuroma OR increased to 1.9, 95 % CI = 0.6-5.9.

In another study by Muscat et al (2002) presented results from a hospital based case-control study on acoustic neuroma on 90 (100 %) patients and 86 (100 %) controls. Cell phone use 1-2 years gave OR = 0.5, 95 % CI = 0.2-1.3 (n=7 cases), increasing to OR = 1.7, 95 % CI = 0.5-5.1 (n=11 cases), in the group with 3-6 years use. Average use among cases was 4.1 years and among controls 2.2 years.

C. Danish cohort study

A population based cohort study in Denmark of mobile phone users during 1982 to 1995 included over 700,000 users (Johansen et al 2001). About 200,000 individuals were excluded since they had company paid mobile phones. Of digital (GSM) subscribers only nine cases had used the phone for ≥ 3 years duration yielding standardised incidence ratio (SIR) of 1.2, 95 % CI = 0.6-2.3. No subjects with 10-year use were reported.

This cohort study was updated with follow-up through 2002 for cancer incidence (Schüz et al 2006). There was no truly unexposed group for comparison since a large part of the population uses wireless phones. Moreover the excluded company subscribers (> 200 000 or 32 %) were apparently included in the reference population. There was also a very skewed sex distribution with 85 % men and only 15 % women in the cohort. SIR was significantly decreased to 0.95, 95 % CI = 0.9-0.97 for all cancers indicating a "healthy worker" effect in the study. In the group with ≥ 10 years since first subscription significantly decreased SIR of 0.7, 95 % CI = 0.4-0.95 was found for brain and nervous system tumours indicating methodological problems in the study. No latency data were given or laterality of phone use in relation to tumour localisation in the brain. This study was uninformative regarding long-term health effects from mobile phone use.

D. Finnish study

Auvinen et al (2002) did a register based case-control study on brain and salivary gland tumors in Finland. All cases aged 20-69 years diagnosed in 1996 were included; 398 brain tumour cases and 34 salivary gland tumour cases. The duration of use was short, for analogue users 2-3 years and for digital less than one year. No association was found for salivary gland tumours. For glioma OR = 2.1, 95 % CI = 1.3-3.4 was calculated for use of analogue phones, but no association was found for digital mobile phones. When duration of use of analogue phones was used as a continuous variable an increased risk was found for glioma with OR = 1.2, 95 % CI = 1.1-1.5 per year of use.

E. The Interphone studies

1. Acoustic neuroma

The Swedish part of the Interphone study on acoustic neuroma included exposure data from 148 (93 %) cases and 604 (72 %) population based controls (Lönn et al 2004). Use of digital phones with time $\geq$ 5 years since first use gave OR = 1.2, 95 % CI = 0.7-2.1. No subjects were reported with use of a digital phone $\geq$ 10 years. An association was found for use of analogue phones yielding for $\geq$ 10 years latency period OR = 1.8, 95 % CI = 0.8-4.3 increasing to OR = 3.9, 95 % CI = 1.6-9.5 for ipsilateral use.

In Denmark the Interphone study included 106 (82 %) interviewed cases with acoustic neuroma and 212 (64 %) population-based controls (Christensen et al 2004). Significantly larger tumours were found among cellular phone users, 1.66 cm^3 compared with 1.39 cm^3 among non-users, p =

0.03. However OR was not significantly increased but only two cases had use a mobile phone regularly $\geq$ 10 years.

Schoemaker et al (2005) presented results for acoustic neuroma as part of the Interphone study performed in 6 different regions in the Nordic countries and UK, as previously partly reported (Lönn et al 2004; Christensen et al 2004). The results were based on 678 (82 %) cases and 3,553 (42 %) controls. Lifetime use of mobile phone for $\geq$ 10 years gave for ipsilateral acoustic neuroma OR = 1.8, 95 % CI = 1.1-3.1, and for contralateral OR = 0.9, 95 % CI = 0.5-1.8.

The study from Japan by Takebayashi et al (2006) included 101 (84 %) acoustic neuroma cases aged 30-69 years and diagnosed during 2000-2004. Using random digit dialling 339 (52 %) controls were interview. No association was found, OR = 0.7, 95% CI = 0.4 – 1.2. No exposure related increase in the risk of acoustic neuroma was observed when the cumulative length of use (<4 years, 4-8 years, >8 years) or cumulative call time (<300 hours, 300-900 hours, >900 hours) was used as an exposure index. The OR was 1.1, 95% CI = 0.6 - 2.1, when the reference date was set to five years before the diagnosis. Further, laterality of mobile phone use was not associated with tumours. No cases with $\geq$10 years latency period were reported.

Use of mobile phones and risk of acoustic neuroma were published from Norway as part of the Interphone study (Klaeboe et al 2007). It included 45 (68 %) acoustic neuroma cases and 358 (69 %) controls. A decreased risk was found with OR = 0.5, 95 % CI = 0.2-1.0. Using different criteria such as duration of regular use, time since first regular use, cumulative use etc 22 additional ORs and CIs were calculated. Time since first regular use for < 6 years gave OR =

1.0, 95 % CI = 0.2-5.7. All 21 other ORs were < 1.0 indicating systematic bias in the study. No case had a latency period of 10 years.

Schlehofer et al (2007) reported results from the German part of the Interphone study on sporadic acoustic neuroma. The study was performed during October 2000 and October 2003. Four study areas were included and cases were aged 30-59 years, but from October 1, 2001 extended to include the age group 60-69 years. They were recruited from hospitals and included 97 (89 %) cases, however, three with trigeminus neuroma. Controls were randomly selected from population registries and in total 202 (55 %) agreed to participate. No association was found for regular mobile phone use, OR = 0.7, 95 % CI = 0.4-1.2. Most ORs were < 1.0 and a decreasing trend of the risk was found for time since first regular use, lifetime number of use and duration of calls. No case had a latency period > 10 years. However, increased OR was found for highly exposed in "specified occupational exposure" yielding OR = 1.5, 95 % CI =0.5-4.2.

E. The Interphone studies

2. Glioma, meningioma

Lönn et al (2005) also studied glioma and meningioma. Data were obtained for 371 (74 %) glioma and 273 (85 %) meningioma cases. The control group consisted of 674 (71 %) subjects. No association was found although time since first regular phone use for $\geq$ 10 years gave for ipsilateral glioma OR = 1.6, 95 % CI = 0.8-3.4 and for contralateral glioma OR = 0.7, 95 % CI = 0.3-1.5.

For ipsilateral meningioma OR = 1.3, 95 % CI = 0.5-3.9 was calculated and for contralateral OR = 0.5, 95 % CI = 0.1-1.7 using 10 $\geq$ years latency period.

The Danish part of the Interphone study on brain tumours (Christensen et al, 2005) included 252 (71 %) persons with glioma, 175 (74 %) with meningioma and 822 (64 %) controls. For meningioma OR = 0.8, 95 % CI = 0.5-1.3 was calculated and for low-grade glioma OR = 1.1, 95 % CI = 0.6-2.0, and for high-grade glioma OR = 0.6, 95 % CI = 0.4-0.9 were found. Use for ≥ 10 years yielded for meningioma OR = 1.0, 95 % CI = 0.3-3.2, low-grade glioma OR = 1.6, 95 % CI = 0.4-6.1 and for high-grade glioma OR = 0.5, 95 % CI = 0.2-1.3. Regarding high-grade glioma 17 ORs were presented and all showed OR < 1.0.

Results from England were based on 966 (51 %) glioma cases and 1,716 (45 %) controls (Hepworth et al 2006). Cases were ascertained from multiple sources including hospital departments and cancer registries. The controls were randomly selected from general practioners' lists. Regular phone use gave OR = 0.9, 95 % CI = 0.8-1.1, increasing to OR = 1.2, 95 % CI = 1.02-1.5 for ipsilateral use but OR = 0.8, 95 % CI = 0.6-0.9 for contralateral use. Ipsilateral use for ≥ 10 years produced OR = 1.6, 95 % CI = 0.9-2.8, and contralateral OR = 0.8, 95 % CI = 0.4-1.4.

Schüz et al (2006) carried out a population-based case-control study in three regions of Germany, with incident cases of glioma and meningioma aged 30-69 years during 2000-2003. Controls were randomly drawn from population registries. In total, 366 (80 %) glioma cases, 381 (88 %) meningioma cases, and 1,494 (61 %) controls were interviewed. For glioma OR = 1.0, 95% CI = 0.7 - 1.3 and for meningioma OR = 0.8, 95% CI = 0.6 - 1.1 were obtained. However, among persons who had used cellular phones for ≥ 10 years increased risk was found for glioma; OR = 2.2, 95% CI = 0.9 - 5.1 but not for meningioma; OR = 1.1, 95% CI = 0.4 – 3.4. Among women they found OR = 2.0, 95 % CI = 1.1-3.5 for high-grade glioma for "regular" cell-phone use.

Summary results for mobile phone use and risk of glioma in Denmark, and parts of Finland, Norway, Sweden and United Kingdom have been published (Lahkola et al 2007). Of the included Interphone studies results had already been published from Sweden (Lönn et al 2005), Denmark (Christensen et al 2005) and UK (Hepworth et al 2006). The results were based on 2,530 eligible cases but only 1,521 (60%) participated. Regular mobile phone use gave OR = 0.8, 95 % CI = 0.7-0.9, but cumulative hours of use yielded OR = 1.006, 95 % CI = 1.002-1.010 per 100 hours. Ipsilateral mobile phone use for $\geq$ 10 years gave OR = 1.4, 95 % CI = 1.01-1.9, *p* trend = 0.04 and contralateral use OR = 1.0, 95 % CI = 0.7-1.4.

Use of mobile phones and risk of glioma and meningioma were published from Norway as part of the Interphone study (Klaeboe et al 2007). It included 289 (71 %) glioma cases, 207 (69 %) meningioma cases and 358 (69 %) controls. Significantly decreased OR = 0.6, 95 % CI = 0.4-0.9 was found for glioma and decreased OR = 0.8, 95 % CI = 0.5-1.1 for meningioma. For glioma 22 additional ORs were calculated using different exposure criteria as discussed above and all calculations yielded OR < 1.0, seven significantly so. Also for meningioma most ORs were < 1.0. Again these results indicate systematic bias in the study.

F. Meta-analysis

A meta-analysis of the risk for acoustic neuroma, glioma and meningioma was performed for mobile phone use with a latency period of 10 years or more (Hardell et al 2007). For acoustic neuroma studies by Lönn et al (2004), Christensen et al (2004) Schoemaker et al (2005) and Hardell et al (2006a) were included, all giving results for at least 10 years latency period or more. Overall OR = 1.3, 95 % CI = 0.6-2.8 was obtained increasing to OR = 2.4, 95 % CI = 1.1-5.3 for ipsilateral mobile phone use (Lönn et al 2004, Schoemaker et al 2005, Hardell et al 2006). For glioma OR = 1.2, 95 % CI = 0.8-1.9 was calculated (Lönn et al 2005, Christensen et al 2005, Hepworth et al 2006, Schüz et al 2006, Hardell et al 2006b, Lahkola et al 2007). Ipsilateral use yielded OR = 2.0, 95 % CI = 1.2-3.4 (Lönn et al 2005, Hepworth et al 2006, Hardell et al 2006b, Lahkola et al 2007). In total OR = 1.3, 95 % CI = 0.9-1.8 was found for meningioma (Lönn et al 2005, Christensen et al 2005, Schüz et al 2006, Hardell et al 2006a) increasing to OR = 1.7, 95 % CI = 0.99-3.1 for ipsilateral use (Lönn et al 2005, Hardell et al 2006b).

IV. Discussion

This review included 20 studies, two cohort studies and 18 case-control studies. We recently made a review on this topic and more details can be found in that publication (Hardell et al 2007). Only two studies have been published since then. Both were on acoustic neuroma (Klaeboe et al 2007, Schlehofer et al 2007). They were small with no cases with a latency period of at least 10 years. Furthermore, most ORs were < 1.0 indicating serious methodological problems in the studies.

So far most studies have had no or limited information on long-term users. No other studies than from the Hardell group has published results for use of cordless phones (Hardell et al 2006a,b). As we have discussed in our publications it is pertinent to include also such use in this type of studies. Cordless phones are an important source of exposure to microwaves and they are usually used for a longer time period on daily basis as compared with mobile phones. Thus, to exclude such use seems to underestimate the risk for brain tumors from use of wireless phones.

It should be noted that the Hardell group has included also use of cordless phones, and thus in the exposure assessment the "unexposed" cases and controls have not been exposed to either cordless or cellular phones. This is in contrast to the Interphone study where the "unexposed" may have been exposed to cordless phones of unknown amount.

Of the 18 case-control studies 11 gave results for ≥ 10 years use or latency period. However, most of the results were based on low numbers. Thus, it is necessary to get an overview if there is a consistent pattern of increased risk with longer latency period and to make a formal meta-analysis of these findings. Since brain tumours are a heterogenic group of tumours it is reasonable to separate the results for malignant and benign tumours, as has been done in the various studies.

The Danish cohort study (Johansen et al, 2001) is not very informative due to limits in study design, analysis and follow-up. Schüz et al. (2006) reported an update of this previous study on mobile phone subscribers in Denmark. Since this report has gained substantial media coverage as "proof" of no brain tumor risk from mobile phone use we will discuss the shortcomings of the study in more detail in the following.

The cohort was established for persons that some time during 1982–1995 were registered cellular telephone users and has now been followed against the Danish Cancer Registry until 2002, seven years more than in the previous study. Previously (Johansen et al, 2001) 9 persons with brain tumors had used GSM phones for > 3 years, and OR =1.2 was reported. Now, data were not provided for type of phone or years of use. Rather the calculation of latency was based on first year of registration.

During early 1980s almost all cellular telephones were used in cars with external antennae. These subjects were unexposed to electromagnetic fields (EMF). No information regarding such use is provided, and one may assume that such participants are now included as exposed although they were not. Over 200 000 (32 %) company subscribers were excluded from the cohort. These are the heaviest users and are billed 4.5 times more than the layman in Sweden. They started use the earliest, but were included in the “non-user” group, i.e., the general Danish population.

SIR among cellular telephone users was 1.21 for temporal glioma (Schüz et al 2006), a region most exposed to EMF, based on 54 persons and not on phone type or time of first use (latency period). No information regarding the ear used and correlation with tumor site was given. The expected numbers were based on the general population. Because a large part of the population uses mobile phones and/or cordless phones, and the latter use was not assessed at all in the study, there is no truly unexposed group for comparison. Risk of cancer was underestimated, e.g., in the group with first use ≥ 10 years, the associated risk for brain tumors was low (SIR =0.7, 95 % CI = 0.4- 0.95). Relying on private cellular network subscription as measure of mobile phone use has been questioned (Ahlbom et al 2004, Funch et al 1996).

There seems to be a “healthy worker” effect in the study because of the decreased overall cancer risk (SIR= 0.9, 95 % CI = 0.9-0.95). Of the subscribers 85 % were men and 15 % women. Certainly early mobile phone users are not socioeconomically representative of the whole Danish population, used for comparison. The cohort only included people > 18 years of age. We reported (Hardell et al 2004, 2006a,b) that cellular telephone use beginning before age 20 is associated with a higher risk of brain tumours than use starting after age 20.

The authors do not acknowledge the contribution by the telecom industry as cited in the first publication (Johansen et al 2001), i.e., TelemarkDanmarkMobil and Sonofom. Two of the authors are affiliated with the private International Epidemiology Institute, Rockville, MD, USA, which has contributed financially to the study. Where the International Epidemiology Institute gets its money from is not declared. In the application to the Danish National Mobile Phone Program, which funded part of the study, no mention of the involvement or payment of these two consultants was made, a fact that is now being set under question.

Regarding the case-control studies there seems to be a consistent pattern of an increased risk for acoustic neuroma using a 10-year latency period and considering ipsilateral exposure. It might be a "signal" tumour type for increased brain tumour risk from microwave exposure, since it is located in an anatomical area with high exposure during calls with cellular or cordless phones (Hardell et al, 2003). Christensen et al (2004) found no association using a ≥ 10 year latency period, but the result was based on only 2 cases. Interestingly, the tumours were significantly larger in the total group of regular mobile phone users.

In our study we found an increased risk also with shorter latency period than 10 years (Hardell et al 2006a). However, it is not known at what stage in the carcinogenesis microwaves act. An effect might exist at different stages both of promoter and initiator type. We conclude that the results on acoustic neuroma are consistent with an association with use of cellular phones using a latency period of ≥ 10 years.

Regarding meningioma no consistent pattern of an association was found, although ipsilateral exposure in the ≥ 10 years latency group increased the risk in the meta-analysis. For a definite conclusion longer follow-up studies are needed. We conclude that the results are not consistent with an association between use of mobile phones and meningioma.

Malignant brain tumours have been studied in 8 case-control studies. One study was register based and showed an increased risk associated with analogue phone use although the latency period seemed to be short (Auvinen et al 2002). The risk of glioma increased significantly per year of use. Five studies gave results for use of cell phone for 10 years or more. The pattern of an association was consistent in the different studies, except for the Danish study by Christensen et al (2005). In that study all 17 odds ratios for high-grade glioma were < 1.0 indicating systematic bias in assessment of exposure.

Our meta-analysis showed a significantly increased risk for ipsilateral use. We conclude that using ≥ 10 years latency period gives a consistent pattern of an association between use of mobile phones and glioma.

Regarding the Interphone studies the German part (Schüz et al 2006) was commented on by Morgan (2006) and these comments may also apply to the other Interphone studies. Morgan noted that the definition of a "regular" cell-phone user was so minimal that almost all "regular" cell-phone users would not be expected to be at risk, even if cell-phone use was found to create very high risks of glioma and meningioma. As for longer periods of "regular" cell-phone use, Schüz et al (2006) reported that only 14 percent of the glioma cases and 6 percent of the meningioma cases had used a cell phone for 5 years or more. For 10 years or more, the percentages were 3 percent and 1 percent, respectively. The authors replied that even long-term users in the study had barely more than 10 years of regular use and, in the beginning, were not heavy users; hence, they could not draw conclusions on heavy long-term use.

Methodological issues in the Interphone studies have been also discussed by Vrijhed et al (2006a,b). It was concluded that actual use of mobile phones was underestimated in light users and overestimated in heavy users. Random recall bias could lead to large underestimation in the risk of brain tumours associated with mobile phone use. According to the authors there was a selection bias in the Interphone study resulting in under selection of unexposed controls with decreasing risk at low to moderate exposure levels. Some of the Interphone studies had a low response rate, especially among controls giving potential selection bias.

A formal meta-analysis on mobile phone use and intracranial tumors was performed by Lahkola et al (2006). No data were given for ≥ 10 year latency period. Overall the risk increased for ipsilateral tumors, OR = 1.3, 95 % CI = 0.99-1.9 whereas no increased risk was found for contralateral tumors, OR = 1.0, 95 % CI = 0.8-1.4.

V. Conclusions

In summary we conclude that our review yielded a consistent pattern of an increased risk for acoustic neuroma and glioma after ≥ 10 years mobile phone use. We conclude that current standard for exposure to microwaves during mobile phone use is not safe for long-term brain tumor risk and needs to be revised.

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Table. Summary of 20 studies on the use of cellular telephones and brain tumour risk. For further details, see Hardell et al (2007). Odds ratio (OR), 95 % confidence interval (CI) and standardised incidence ratio (SIR) are given.

Study	**Years Study Type**	**Age**	**Tumour type**	**No. of Cases**	**Odds ratio, 95 % confidence interval**	**Comments**
Hardell et al 1999, 2001 Sweden	1994-1996 Case-control	20-80 years	Brain tumours	217	OR 1.0 (0.7-1.4)	Analogue and digital cell phone use
				34	OR 1.1 (0.6-1.8)	Ipsilateral
				16	OR 1.2 (0.6-2.6)	> 10 year latency, analogue cell phone
Muscat et al 2000 USA	1994-1998 Case-control	18-80 years	Brain tumours	17	OR 0.7 (0.4-1.4)	Mean duration of use, 2.8 years
			Neuorepithelioma	35	OR 2.1 (0.9-4.7)	
Johansen et al 2001 Denmark	1982-1995 Cohort	0 to > 65 years	Brain tumours	20	SIR 1.3 (0.8-2.1)	Analogue and digital cell phone use
				9	SIR 1.2 (0.6-2.3)	≥ 3 years duration of digital subscription
Inskip et al 2001 USA	1994-1998 Case-control	≥ 18 years	Acoustic neuroma	5	OR 1.9 (0.6-5.9)	≥ 5 years of cell phone use
			Glioma	11	OR 0.6 (0.3-1.3)	
			Meningioma	6	OR 0.9 (0.3-2.7)	
Muscat et al 2002 USA	1997-1999 Case-control	≥ 18 years	Acoustic neuroma	11	OR 1.7 (0.5-5.1)	3-6 years of cell phone use
Auvinen et al 2002 Finland	1996 Case-control, register based	20-69 years	Glioma	119	OR 1.5 (1.0-2.4)	Analogue and digital cell phone ”ever” use
				40	OR 2.1 (1.3-3.4)	Analogue cell phone ”ever” used
				11	OR 2.4 (1.2-5.1)	Analogue cell phone use 1-2 years
				11	OR 2.0 (1.0-4.1)	Analogue cell phone use, >2 years
Lönn et al 2004 Sweden Interphone	1999-2002 Case-control	20-69 years	Acoustic neuroma	12	OR 1.8 (0.8-4.3)	≥10 years of cell phone use, result for either side of head
				12	OR 3.9 (1.6-9.5)	≥10 years of cell phone use on same side of head as tumour

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Christensen et al 2004 Denmark Interphone	2000-2002 Case-control	20-69 years	Acoustic neuroma	45	OR 0.9 (0.5-1.6)	Regular use
				2	OR 0.2 (0.04-1.1)	≥ 10 years cell phone use on same side of head as tumour. Significantly larger tumours among cellular phone users 1.66 cm^3 *versus* 1.39 cm^3, p=0.03.
Lönn et al 2005 Sweden Interphone	2000-2002 Case-control	20-69 years	Glioma	214	OR 0.8 (0.6-1.0)	Regular use
				15	OR 1.6 (0.8-3.4)	≥10 years since first "regular" cell phone use on same side of head as tumour
				11	OR 0.7 (0.3-1.5)	≥10 years since first "regular" cell phone use on opposite side of head as tumour.
			Meningioma	118	OR 0.7 (0.5-0.9)	Regular use
				5	OR 1.3 (0.5-3.9)	≥10 years since first "regular" cell phone use on same side of head as tumour
				3	OR 0.5 (0.1-1.7)	≥10 years since first "regular" cell phone use on opposite side of head as tumour.

Study	**Years Study Type**	**Age**	**Tumour type**	**No. of Cases**	**Odds ratio, 95 % confidence interval**	**Comments**
Schoemaker et al 2005 Denmark, Finland, Sweden, Norway, Scotland, England, Interphone	1999-2004 Case-control	18-69 years (variable)	Acoustic neuroma	360	OR 0.9 (0.7-1.1)	Regular use
				23	OR **1.8** (1.1-3.1)	≥ 10 lifetime years of cell phone use on same side of head as tumour
				12	OR 0.9 (0.5-1.8)	≥ 10 lifetime years of cell phone use on opposite side of head as tumour
Christensen et al 2005 Denmark Interphone	2000-2002 Case-control	20-69 years	Low-grade glioma	47	OR 1.1 (0.6-2.0)	Regular use
				9	OR 1.6 (0.4-6.1)	≥10 years since first regular use of cell phone
			High-grade glioma	59	OR 0.6 (0.4-0.9)	Regular use
				8	OR 0.5 (0.2-1.3)	≥10 years since first regular use of cell phone 17 odds ratios for high-grade glioma, all < 1.0, indicates systematic bias
			Meningioma	67	OR 0.8 (0.5-1.3)	Regular use
				6	OR 1.0 (0.3-3.2)	≥10 years since first regular use of cell phone
Hepworth et al 2006 UK Interphone	2000-2004 Case-control	18-69 years	Glioma	508	OR 0.9 (0.8-1.1)	Regular use
				NA	OR 1.6 (0.9-2.8)	≥10 years of cell phone use on same side of head as tumour.
				NA	OR 0.8 (0.4-1.4)	>10 years of cell phone use on opposite side of head as tumour.

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Schüz et al 2006 Germany Interphone	2000-2003 Case-control	30-59 years	Glioma	138	OR 1.0 (0.7-1.3)	Regular use
				12	OR 2.2 (0.9-5.1)	≥ 10 years since first regular use of cell phone
				30	OR **2.0** (1.1-3.5)	Female regular use of cell phone
			Meningioma	104	OR 0.8 (0.6-1.1)	Regular use
				5	OR 1.1 (0.4-3.4)	≥ 10 years since first regular use of cell phone

Hardell et al 2006a Sweden	1997-2003 Case-control	20-80 years	Acoustic neuroma	130	OR **1.7** (1.2-2.3)	> 1 year latency of cell phone use
				20	OR **2.9** (1.6-5.5)	> 10 years latency of cell phone use
				10	OR **3.5** (1.5-7.8)	> 10 years of ipsilateral cell phone use
				4	OR 1.0 (0.3-2.9)	> 10 years latency of cordless phone use
				3	OR 3.1 (0.8-12)	> 10 years latency of ipsilateral cordless phone use
			Meningioma	347	OR 1.1 (0.9-1.3)	> 1 year latency of cell phone use
				38	OR 1.5 (0.98-2.4)	> 10 years latency of cell phone use
				15	OR 2.0 (0.98-3.9)	> 10 years latency of ipsilateral cell phone use
				23	OR 1.6 (0.9-2.8)	> 10 years latency of cordless phone use
				9	OR **3.2** (1.2-8.4)	> 10 years latency of ipsilateral cordless phone use
Hardell et al 2006b Sweden	1997-2003 Case-control	20-80 years	Glioma, high-grade	281	OR **1.4** (1.1-1.8)	> 1 year latency of cell phone use
				71	OR **3.1** (2.0-4.6)	> 10 years latency of cell phone use
				39	OR **5.4** (3.0-9.6)	> 10 years latency of ipsilateral cell phone use
				23	OR **2.2** (1.3-3.9)	> 10 years of cordless phone use
				10	OR **4.7** (1.8-13)	> 10 years latency of ipsilateral cordless phone use
			Glioma, low-grade	65	OR 1.4 (0.9-2.3)	> 1 year latency of cell phone use
				7	OR 1.5 (0.6-3.8)	> 10 years latency of cell phone use
				2	OR 1.2 (0.3-5.8)	> 10 years latency of ipsilateral cell phone use
				5	OR 1.6 (0.5-4.6)	> 10 years latency of cordless phone use
				3	OR 3.2 (0.6-16)	> 10 years latency of ipsilateral cordless phone use

Study	Years Study Type	Age	Tumour type	No. of Cases	Odds ratio, 95 % confidence interval	Comments
Takebayashi et al 2006 Tokyo Interphone	2000-2004 Case-control	30-69 years	Acoustic neuroma	51	OR 0.7 (0.4-1.2)	Regular use
				4	OR 0.8 (0.2-2.7)	Length of use > 8 years
				20	OR 0.9 (0.5-1.6)	Ipsilateral use
Schüz et al 2006 Denmark	1982-2002 Cohort	>18 years	Glioma	257	SIR 1.0 (0.9-1.1)	420 095 telephone subscribers
			Meningioma	68	SIR 0.9 (0.7-1.1)	
			Nerve sheat tumors	32	SIR 0.7 (0.5-1.0)	
			Brain and nervous system	28	SIR 0.7 (0.4-0.95)	Latency ≥ 10 years
Lahkola et al 2007 Denmark, Norway, Finland, Sweden, UK Interphone	September 2000-February 2004 (differed between countries) Case-control	20-69 years (Nordic countries), 18-59 years (UK)	Glioma	867	OR 0.8 (0.7-0.9)	Regular use
				77	OR 1.4 (1.01-1.9)	Ipsilateral mobile phone use, ≥ 10 years since first use, *p* for trend = 0.04
Klaeboe et al 2007 Norway Interphone	2001-2002 Case-control	19-69 years	Glioma	161	OR 0.6 (0.4-0.9)	Regular use
			Meningioma	111	OR 0.8 (0.5-1.1)	
Schlehofer et al 2007 Germany Interphone	2000-2003 Case-control	30-69 years	Acoustic neuroma	29	OR 0.7 (0.4-1.2)	Regular use

SECTION 11 - part 2

Evidence for Brain Tumors
(EPIDMIOLOGICAL)

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I. INTRODUCTION

Primary central nervous system (CNS) tumors are a heterogeneous group of benign and malignant neoplasms localized in the brain, the spinal cord and their coverings. They differ in histological type, tissue of origin, anatomic site, growth pattern, age distribution, sex ratio, clinical appearance and many other features including molecular neuropathological markers. These features are not independent but little is known about the etiology of these tumors and the reason for the observed epidemiological patterns. The rapidly developing field of molecular neuropathology may provide clues to solve these problems in the future.

Brain tumors, accounting for the majority of CNS tumors, are rare. Annually about 36,000 36000 new cases are diagnosed in the US and about 180,000 180000 world-wide. The age distribution has two peaks: incidence is about 35 cases per million per year below 10 years of age (which is mainly due to tumors originating from mesodermal and embryonic tissues, medulloblastoma and astrocytoma of the juvenile pilocytic type), and after age 15 there is a steady increase of incidence with increasing age reaching its second peak of about 200 cases per million per year at an age around 75 years. The burden of CNS cancers is distinctly higher in children making up around 20% of all childhood malignancies, while in adults less than 2% of all cancers are primary brain cancers.

There are some rare cases of inherited cancer syndromes (e.g. von Hippel-Lindau disease, Li-Fraumeni syndrome) that are related to brain tumor risk, accounting for a small fraction of cases. Except for therapeutic x-rays no environmental or lifestyle life-stile factor has unequivocally been established as risk factor for brain tumors. Non-whites Non whites seem to have lower risk, and incidence tends to be higher with increasing socio-economic status. However, because of the rather advanced age of 75 of peak incidence, such differences may partly be due to differences in life-expectancy. During the last decades some types of brain tumors show a steady increase of a few percent per year, which might to some extent be related to the introduction of computed tomography and other high-resolution neuroimaging methods.

Since the report of Wertheimer and Leeper in 1979 of an increased incidence of brain tumors in children living in homes with an expected higher exposure to power-frequency electric and magnetic fields, exposure to electromagnetic fields have become an area of interest in the study of factors affecting brain tumor risk.

This review focuses on the radio frequency (RF) part of the electromagnetic spectrum (3 kHz to 300 GHz). However, because the epidemiology of mobile phone use is covered in another section, it will be restricted to RF exposure conditions other than microwaves from mobile phone use. Exposure to ELF magnetic fields and childhood brain tumors is covered in the chapter about childhood cancers.

II. Material and Methods

Published articles of relevant studies restricted to the last 20 years were obtained by searching PubMed using the following terms:

("radio frequency" OR electromagnetic* OR microwaves) AND ("brain cancer" OR brain tumor* OR "CNS cancer" OR CNS tumor* OR glioma* OR meningioma* OR neuroma*) NOT ("power frequency" OR "low frequency") AND epidemiology

The search resulted in 101 hits. After removing reviews and animal or in vitro studies as well as studies of mobile phone use, 8 articles remained. A hand search in review papers (Krewski et al. 2001; Elwood 2003; Ahlbom et al. 2004; Kundi et al. 2004) and reference lists of the articles found in PubMed revealed another 7 papers; hence the final body of evidence consists of 15 studies of exposure to various types of RF fields.

Of the 15 studies 8 were cohort studies, 3 case-control studies and 4 of an ecological type. The majority (11) were occupational studies, two studies investigated children, and one ecological study investigated adults and one study both, adults and children.

III. Epidemiological studies of RF fields and brain tumors

Table 1 gives an overview of the 15 studies obtained by the literature search with respect to study type, assessment of exposure and outcome, confounders considered and matching variables used, number of cases included and selection method of study participants. Results are summarized in Table 2.

In the following paragraphs each study is briefly discussed with respect to its strengths and weaknesses.

A. Thomas et al. 1987

This case-control study included 435 deaths from brain or CNS tumors and 386 deaths from other causes as controls. Only adult males were included. Basis of data collection on occupational history were interview with next-of-kin. Two methods of classification were used: one method assigned subjects to one of three categories (never exposed to RF/ever exposed to RF in an electrical or electronics job/ever exposed to RF but not in an electrical or electronics job), the other method consisted in a classification of each job by an industrial hygienist hyginiest for presumed exposure to RF, soldering fumes, and lead. Both methods revealed significantly increased brain tumor risks of presumed occupational exposure to RF fields. This increase was due to an association in electronics and electrical jobs with astrocytic tumors as the predominant outcome associated with employment in these categories. In addition a significant increase of brain tumor risk was found for increasing duration of exposure.

Although relying on information of next-of-kin could be a source of misclassification, one strength of this study is it's its relying on occupational history only that could be assumed to be more accurate than recall of exposure to various agents. The two methods of classification led to almost the same results, which lends support to the hypothesis that indeed exposure in electrical and electronics jobs is associated with an increased brain tumor risk. Due to the strong relationship between RF exposure and exposure to lead, solvents or soldering fumes in these jobs, it is not possible to separate effects of these exposures. However, analysis of exposure to lead did not show a consistent relationship with brain tumor risk, indicating that it may not confound the relationship to RF exposure.

Because this study is of dead cases only it is likely over-representing high grade brain tumors that may not all be associated with exposure which leads to an effect dilution. Exposure misclassification, if it is non-differential in cases and controls, also tends to reduce effect estimates.

A weakness of this study is obviously its lack of an exposure indicator other than the occupational category. While there is no doubt that in these jobs some exposure to RF fields occur quite regularly, specific characteristics including frequency ranges, modulation, intensity, duration and distance from the source vary considerably. Overall the study (as well as two earlier ones outside the search window: Lin et al. 1985 and Milham 1985) are sufficient to formulate a research hypothesis that can be tested in appropriately designed subsequent investigations. Unfortunately such studies have never been conducted.

B. Milham 1988

In this cohort study of 67,829 amateur radio operators holding a license within 1/1979 to 6/1984 in Washington and California 29 brain tumor deaths occurred during the follow up period with 21 expected.

It should be noted that there was a substantial and statistically significant lower number of overall deaths of less than three quarters of deaths expected from country mortality rates. This could be due to both a 'healthy-worker' effect as well as an effect of socio-economic status. In lieu of computing standardized mortality ratios (SMR) it may be instructive to look at the proportional mortality rates in the reference population and the amateur radio operators: 0.6% of all deaths are expected to be due to brain tumors in the reference population while in amateur radio operators twice as many occurred (1.2%). Whether or not this is an indication of an increased brain tumor risk due to RF exposure is difficult to assess. First of all this study is a register only investigation and no information on intensity, frequency and duration of engagement in amateur radio operations are available. In a later analysis the author reported about results using a proxy of intensity and duration of exposure: the license class. In this analysis indications of an increase of risk with increasing license class were obtained.

This study could and should have started off a thorough follow up of amateur radio operators and nested case-control studies to address the problem of potential confounders and to narrow down the conditions that may be responsible for the increased mortality from some cancers. It is another loose end that leaves us without a clear message.

Although no risk factor for brain cancer except therapeutic ionizing radiation is known, there are some indications that risk increases with social class. The reason for this association is unknown but life-style factors may play a role as well as concomitant causes of death that could lead to a spurious reduction of risk in lower class populations because brain tumors have their peak close to life-expectancy.

C. Selvin et al. 1992

The objective of this investigation was not primarily to study the relationship between RF exposure and childhood cancer but to address the general problem of how to assess disease incidence or mortality in relation to a point source. As the point source the Sutro Tower in San Francisco, the only microwaves emitting tower in this county, was chosen. A total of 35 brain tumor deaths occurred among 50,686 white individuals at risk aged less than 21 in the years 1973-88 in an area of approximately 6 km around the tower. The exact location of residence could not be obtained; therefore each case was located in the center of the census tract. Different methods of analysis were applied to assess a potential relationship between distance from the tower and brain tumor risk. Relative risk for brain tumors for a distance less than 3.5 km from Sutro Tower compared to more than 3.5 km was 1.162 and not significant.

The study explored different methodological procedures and has its merits from a methodological point of view. However, it starts from the wrong assumption: that distance to a point source is a valid proxy for intensity of exposure. Under ideal conditions of spherical symmetry of an emission this assumption holds, however, there are almost no real life situations where this assumption is sufficiently close to actual exposure levels. And it is definitely not true for the Sutro Tower. Radiations from the antennae are directed towards the horizon and the complex pattern of emission with main and side lobes results in a complex pattern of RF exposure at ground level. Furthermore, the area is topographically structured with hills and valleys such that areas of high exposure at the vertices are in close proximity to areas of low exposure at the shadowed side downhill.

Studying the relationship between a point source and disease is not only difficult due to the complex relationship between distance and exposure but also because of the fact that humans are not stable at a certain location. This is of greater importance for adults who may commute from and to work places and have generally a greater radius of activity as compared to children. Nevertheless, there is at least a high chance of one long-lasting stable location that is when people sleep in their beds. Therefore, studies in relation to a point source should attempt to assess exposure at the location of the bed. Because the objective of this study was not the assessment of a potential brain tumor risk but the application of methods for the analysis of spatial data, no attempts were made to measure actual exposure.

D. Tynes et al. 1992

In this study information on occupations obtained for all Norwegians every 10 years was used to assess cancer incidence in relation to job titles. In 1960 37,945 male workers were identified that had jobs with possible exposure to EMFs and among these 3,017 with possible RF exposure. Overall 119 brain tumor cases were found in the cancer registry between 1961 and 1985. Of these cases 6 occurred in the subgroup of workers possibly exposed to RF fields. The overall expected number of brain tumor cases was 109 and 12 for the subgroup with possible RF exposure. Hence no increased brain tumor risk could be detected.

Despite the long follow-up period of 25 years with an accumulated number of 65,500 person-years the expected number of brain tumors diagnosed during that period is too low to detect a moderately elevated risk of 1.3 to 1.5.

As mentioned above, all studies solely relying on job titles lead to exposure misclassification and, therefore, to a dilution of risk. For dichotomous exposure variables (exposed/not exposed) and assuming a negligibly small proportion of exposed in the reference population standardized incidence ratios (SIR) are biased by a factor $(1+f*(SIR-1))/SIR$, if f denotes the fraction of true exposed and SIR is the true incidence ratio. Hence a true SIR of 2.0 is reduced to 1.5 if only 50% in the cohort are actually exposed. The observed SIR is further reduced if the assumption of a negligible fraction of exposed in the reference population is wrong. In this case the bias factor given above is further divided by $(1+g*(SIR-1))$, where g is the fraction of exposed in the general population.

While a cohort study that is based on registry data has the advantage of independence from recall errors and selection bias due to possible differential participation, it has the disadvantage that registry data are generally insufficient to provide reliable exposure indicators. While no association with brain tumors could be detected in this study it revealed an increased number of leukemia cases in occupations with possible RF exposure. This could be due to the higher incidence of leukemia or to a stronger association or to different latency periods and various other reasons including chance.

E. Grayson 1996

In this case-control study nested within approx. 880,000 US Air Force personnel with at least one years of service during the study period of 1970-89 primary malignant brain tumor cases were ascertained by screening hospital discharge records. The study included only males and only as long as they were on Air Force records. From 246 cases detected 16 were dropped due to incomplete or ambiguous data. For each case four controls were randomly selected from the case's risk set matching it exactly on year of birth and race. Controls who were diagnosed with diseases that may be associated with EMF exposure (leukemia, breast cancer, malignant melanoma) were excluded from the risk set.

One strength of this study is the detailed job history filed for each cohort member that could be used for retrospective exposure assessment. Furthermore, Air Force files contained detailed data from personal dosímetry on ionizing radiation for the different posts and jobs. Classification of RF field exposure was based on a detailed job exposure matrix with over 1,950 entries, indexing 552 different job titles. One source of classification was recorded events of exposure to RF fields above 100 W/m2. By this method probable exposure was assigned if for a job such events were recorded in the past as well as for closely related jobs. Possible exposure was assigned for jobs that required operation of RF emitters but without recorded overexposure.

A further strength is the thorough consideration of possible confounders. Because of the possible relationship of brain tumor risk with socio-economic status (SES), military rank was used as a surrogate for SES and included in the analysis as well as ionizing radiation exposure that has previously been shown to increase brain tumor risk.

Exposure to RF fields was associated with a moderate but statistically significant increased risk of OR=1.39. Investigation of duration of exposure was compromised by an ambiguity introduced by the calculation of an exposure score as the product of exposure and months.

Nevertheless, for those ever exposed there were indications of an increasing risk with increasing exposure duration.

A weakness of this investigation is its incomplete follow-up of cohort members. This could have resulted in an underestimation of the true risk. Leaving the Air Force could have been more likely in those exposed to RF fields and developing a brain tumor. Some malignant brain tumors have early signs that could be incompatible with the Air Force job especially if involving operation of RF equipment (like seizures, severe headaches, somnolence, and absences). Because the study did not involve personal contact it is free of other selection biases.

F. Szmigielski 1996

In this military cohort study of cancer morbidity Polish military career personnel was assessed for occupational exposure to RF fields based on service records. The study covered 15 years (1971-85) including approx. 128,000 persons per year. Expected rates for 12 cancer types were calculated based on the age specific morbidity in those classified as unexposed.
For brain and nervous system tumors a significantly increased ratio of observed to expected (OER=1.91) was found. Other malignancies with significantly increased incidence in exposed were: esophageal and stomach cancers, colorectal cancers, melanoma, and leukemia/lymphoma.

One strength of this study is its substantial size with almost 2 million person-years of follow-up. Furthermore, accurate military records on job assignment and on exposure from military safety groups gives a unique opportunity to assess long-term exposure effects based on already filed data.

Some important data are missing because they were military classified information that could not be provided in the paper. This includes the exact number of cases of the different neoplasms. However, from the data presented an observed number of brain tumors of about 46 can be calculated.

The study has been criticized for an alleged bias because more information on risk factors was available for cancer cases. It is true that military medical boards collected data for cases such as life style factors and exposure to possible carcinogens during service, however, at no stage this information entered the analysis. Therefore, this criticism is unfounded. Such information could have been utilized within a nested case-control study applying the same methods of assessment of risk factors for controls as has been done for cases. Because some findings, such as the increased risk for esophagus/stomach cancer, that are rarely reported in relation to RF exposure warrant further study, such a nested case-control approach is recommended. It could, albeit with some difficulties, even be successfully conducted retrospectively.

G. Hocking et al. 1996

In an ecological study cancer incidence and mortality in nine municipalities of northern Sydney during 1972-90 three of which surround three TV towers were assessed. Population size in the three municipalities located within a radius of approximately approx. 4 km around the TV towers amounts to 135,000 while population size in the six municipalities further away was 450,000. High-power transmission commenced in 1956, an additional 100 kW transmission started in 1965 and another 300 kV broadcast in 1980. Carrier frequencies varied between 63 and 533 MHz for TV broadcasting and was around 100 MHz for FM radio broadcast.

During the study period 740 primary malignant brain tumors were diagnosed in adults and 64 in children, 606 deaths due to brain cancer occurred in adults and 30 in children. While incidence of lymphatic leukemia was significantly higher in adults as well as in children inhabiting the three municipalities surrounding the transmission towers compared to the six districts further away, brain tumor incidence was not significantly elevated (RR=0.89 in adults and 1.10 in children).

As has been stated above, distance from a transmitter is a poor proxy for exposure. Some measurements done in the study area obtained levels much lower than those calculated from the emission power and antenna gain. Several factors are responsible for this effect: multiple reflections, attenuation by buildings and vegetation, ground undulations, non-coincidence of maxima for the different signals as well as complex radiation characteristics of the broadcast antennae.

The exact location of the residence of cases could not be provided which reduces the potential of the study to relate incidences to measurements or calculations of RF fields. Authors discussed some potential sources of bias such as migration and other exposures in the different regions. However, the most important disadvantage in such studies is that individual risk factors cannot be adjusted for. Both spurious positive as well as false negative results can be obtained by disregarding such individual variables.

H. Tynes et al. 1996

In a historical cohort study 2,619 Norwegian female radio and telegraph operators certified between 1920 and 1980 were followed from 1961 through 1991 for entries in the cancer registry. During this period a total of 140 cases of cancer occurred which are about 20% more than expected from the Norwegian population. Among these were 5 brain tumor cases closely matching the number expected.

An excess for breast cancer was found in this study that may be related to a combination of RF field exposure and night work. For other cancers including brain cancer numbers of cases were too low to address exposure risk.

In this very thoroughly conducted study including a nested case-control approach for breast cancer, measurements at historical transmitters on ships, comparison with women at other jobs on sea, brain tumors were not distinctly higher than expected from the reference population. However, because of the limited cohort size a moderately increased risk cannot be excluded.

I. Dolk et al. 1997a

This ecological small area study of cancer incidence 1974-86 near the Sutton Coldfield TV/radio transmitter at the northern edge of the city of Birmingham (England) was initiated by an unconfirmed report of a ‘cluster’ of leukemias and lymphomas. The transmitter came into service in 1949. Transmission at 1 megawatt (effective radiated power erp) began in 1964, at 3 MW in 1969, and at 4 MW in 1982. The tower has a height of 240 m with no big hills in the surrounding area. The study area was defined by a circle of 10 km radius centered at the transmitter. The population within this area was about 408,000. All cancers, excluding non-melanoma skin cancer, were considered focusing on hematopoietic and lymphatic cancers, brain and nervous system cancers, eye cancer, and male breast cancer. Childhood cancers were restricted to all cancers and all leukemias.

In the study area a small but significant excess of all cancers was observed in adults. All leukemias and non-Hodgkin's lymphoma were particularly elevated and incidence within 2 to 4 km from the tower was about 30% higher than expected. Brain tumors were only analyzed for distances of within 2 km and the whole study area. Within 2 km an increased OER of 1.29 for all brain tumors and 1.31 for malignant brain tumors was calculated based on 17 and 12 cases, respectively.

Also this investigation suffers from using distance from the tower as proxy for intensity of exposure. The wrong assumption that exposure decreases with increasing distance invalidates the statistical trend test applied. Measurements conducted in the study area revealed the poor relationship with distance but without consequences on the evaluation of the data. Overall the study is consistent with a moderately increased risk of hematopoietic and lymphatic cancers as well as some other cancers including brain cancer in the vicinity of high-power transmitters that, if related to RF fields, must be substantially higher for actual exposure.

The Sutton Coldfield study was later continued (Cooper & Saunders 2001) to cover the period 1987-94. The study revealed, compared to the earlier period, an almost unchanged increase of leukemias and non-Hodgkin's lymphoma in adults and a slight increase in children.

J. Dolk et al. 1997b

Because the Sutton Coldfield study was triggered by a cluster report and to provide independent test of hypotheses arising from that study, similar methods as applied in the previous study were used to study all high-power TV/radio transmitters (≥ 500 kW ERP) in Great Britain. In adults leukemias, bladder cancer, and skin melanoma, and in children, leukemias and brain tumors were studied. The study period was 1974-86 for England and somewhat shorter in Wales and Scotland.

Although population density around transmitters was not always as high as in the case of the Sutton Coldfield tower, with an average population density of only about one third of that

around Sutton Coldfield tower within 2 km from the towers, in the most important range of 2 to 4 km from the transmitters, where in many cases the maximum of radiated RF at ground level is reached, population density was similar. The study of all high-power transmitters essentially corroborated the findings for adult leukemias with an increase of incidence between 10 and 50% in the distance band of 2 to 4 km from the transmitters for the different transmitter types. Most of these increased incidences were statistically significant.
For children only the incidence in the whole study area and within a distance of 2 km was calculated, which is unfortunate because the area close to the towers is sparsely populated and exposure is low. Number of brain tumors in children was slightly above expectation (244 observed and 231 expected).

In contrast to the interpretation by the authors, the study of all high power transmitters essentially replicated and supported the findings of an excess incidence of leukemias in relation to RF emission from TV/radio towers. Because the different heights and radiation characteristics of the transmitters result in different exposure patterns at ground level, the consistent increase in an area that is likely close to the maximum of exposure supports the hypothesis of an association.

K. Lagorio et al. 1997

A mortality study of a cohort of 481 female plastic-ware workers employed between 1962-92 in an Italian plant, 302 of which were engaged in the sealing department with exposure to RF fields, was reported by Lagorio et al. (1997). For RF-sealers 6,772 person-years of follow-up were accumulated and overall 9 deaths occurred, 6 of which were from malignant neoplasms (which are twice as many as expected from comparison with the local reference population). In the 31 years only one brain cancer occurred but only 0.1 were expected.

Although the small size of the cohort and the potential exposure to other agents except RF fields such as solvents and vinyl chloride prohibit far reaching conclusion, much more of such thorough follow-up studies of exposed cohorts are needed to accumulate a body of evidence that can provide a useful basis for analysis.

L. Finkelstein 1998

A preliminary study intended to form the basis for an assessment of cancer risks associated with handheld radar devices was conducted among a cohort of 20,601 male Ontario police officers. The retrospective follow up covered the period of 1964-95. By linkage with the cancer registry and mortality database 650 cases of cancer were detected.
Testicular cancer and melanoma showed an excess incidence while overall cancer incidence was reduces as expected from a working cohort. Overall 16 cases of primary malignant brain tumors occurred which are slightly less than expected.

The author had difficulties to build up a proper cohort because some departments refused to participate and others couldn't spare the time to provide lists of all officers employed during the target period. Furthermore, while cancer sites of primary interest showed actually an increased incidence calling for a nested case-control approach, this study was never conducted due to lack of interest and support of the authorities.

M. Morgan et al. 2000

In an occupational cohort study all US Motorola employees with at least 6 months cumulative employment and at least 1 day of employment in the period 1976-96 were included. A total of 195,775 workers contributing about 2,7 million person-years were available for the study. The cohort was compared to the SSA Master Mortality File and the National Death Index to obtain vital status. Death certificates were obtained by states' vital statistics offices and company records. Exposure was assessed by expert opinion. Four RF exposure groups were defined with increasing level of estimated RF exposure. Only about 5% of the total cohort was classified as highly exposed and more than 70% with only background exposure. Neither private nor occupational mobile phone use was included.

Overall 6,296 deaths occurred in the cohort in 21 years, which were only two thirds of deaths expected from mortality data of the four countries where most Motorola facilities are located. This reduction is too pronounced to be solely due to a healthy worker effect, other factors such as higher SES must have contributed, an interpretation supported by the substantial reduction of mortality from all life-style associated causes of death. Internal comparisons were done for mortality from brain cancer and hematopoietic and lymphatic cancers. Brain tumor mortality was slightly but insignificantly elevated in high and moderately high exposed workers as compared to those with no or low RF exposure.

This study of a huge cohort demonstrates the limitations of such a study design. The majority of the cohort (58%) consisted of retired or terminated workers that may or may not accumulate further RF exposure at other companies. Furthermore, it can be assumed that Motorola employees were among the first that used mobile phones at the workplace and privately. Neglecting mobile phone use may diminish the gradient of exposures between occupational groups studied. It would have been better to conduct nested case-control studies instead of using internal comparison that may be compromised by mobility bias, exposure misclassification and other sources of bias.

N. Groves et al. 2002

In this military cohort study of 40,581 men followed from the year of graduation (1950-1954) from Navy technical schools through 1997, known as the Korean War Veterans study, groups of sailors with imputed difference in likelihood and amount of exposure to radar waves were compared with respect to mortality. The original study, with a follow up through 1974, (Robinette et al. 1980) reported increased risks of cancer of the hematopoietic and lymphatic system, of the lung and digestive system for the high exposure group but was handicapped by the lack of information on date of birth of the cohort members. For the extended follow up study many missing birth dates were found in the Veterans Administration Master Index. Nevertheless, birth date remained unknown for over 8% of the cohort. Based on expert opinion low RF exposure was assigned to job classifications of radioman, radarman, and aviation electrician’s mate, high exposure stratum included men with job classifications of electronics technician, aviation electronics technician, and fire control technician.

By matching against the Social Security Administration’s Death Master File and the National Death Index 8,393 deceased subjects were identified through 1997. This number is substantially and significantly lower as expected from the male white US population. A healthy soldier effect may have been responsible for a lower mortality rate in the 1950ies but cannot explain the reduced mortality after 40 years. It has not been reported how long the cohort members stayed in service nor were life-style factors investigated; however, of more than 40% of the cohort no social security number could be obtained suggesting possible under-estimation of deaths.

Comparison of high- with low-exposure groups revealed significantly lower mortality from life-style associated causes of death (lung cancer, vascular diseases, diabetes mellitus, chronic obstructive pulmonary disease, liver cirrhosis) and significantly higher mortality from all leukemias and external causes of death. Increased mortality from leukemias was found in all high exposure groups but the most pronounced increase was observed in aviation electronics technicians. Brain cancer was less frequent in all high exposure groups compared to the low exposure category.

The long period of follow up of this large cohort with start of follow up almost at the same time (1950-54) and at a time when exposure commenced is a great advantage of this investigation. However, there are a number of shortcomings: follow up was possibly incomplete by unknown social security number of a substantial proportion of the cohort; almost half of all deaths in the first 20 years were from external causes which could have obscured an effect of exposure; duration and intensity of exposure is unknown as well as potential exposure after leaving the Navy; classification into low and high exposure groups may introduce substantial misclassification. In the earlier report, inspection of Navy records for a sample from the high exposure group revealed that 24% had no exposure to radar waves at all.

Concerning brain tumors, assuming an effect of radar exposure on growth rate, exposure during the Korean War and no exposure afterwards would be expected to result in only a slightly increased risk during a period of about 10 years after the war. Sailors were about 20 to 25 years at that time. The fraction with an already initiated brain tumor during this age range is estimated to be less than 3 in 100,000 per year. Increase of growth rate even if substantial cannot result in an effect observable in a cohort of that size. If radar exposure increases the likelihood of malignant transformation this could increase the incidence during a time window of 10 to 20 years after the exposure period. Results of the Israeli study of x-ray treated tinea capitis (Sadetzki et al. 2005) suggest an even longer latency, however, risk decreased with increasing age at first exposure to x-rays. In addition, for malignant brain tumors there is a less pronounced relationship to ionizing radiation, and a higher risk was observed for meningioma that were not investigated in the Korean War Veterans study. Taking the data on ionizing radiation as a guiding principle for brain tumor initiation, radar exposure of sailors during their twenties might result in an increase of brain tumor mortality of about 10 to 15%, i.e. a maximum of 8 additional cases among 20,000. Considering the biases of the study such a low risk is easily obscured. Hence neither tumor promotion nor initiation may be detected in this study even if there is an increased risk. Because of the mentioned limitation to a certain time window with possibly increased incidence due to exposures during service in the Korean War, it would have been instructive to compute Kaplan-Meier estimates for cumulative brain tumor mortality.

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In the German part of the Interphone study special attention was paid to occupational history and exposure to RF fields at workplaces. Incident meningioma (n=381, response rate 88%) and glioma cases (n=366, response rate 80%) aged 30-69 years were selected from four neurological clinics. Overall 1,535 (participation rate 63%) were randomly selected from population registries matched to the cases by sex, age, and region. Most cases were interviewed during their stay in hospitals, controls were interviewed at home. The interview contained several screening questions about occupations that are probably associated with RF exposure. If any of these screening questions were marked additional questions were asked about the job. Based on the literature and the evaluation by two industrial hygienists a classification into the following categories was performed: no RF exposure/not probably RF exposed/probably ER exposed/highly RF exposed. In total about 13% (299 cases and controls) were classified with at least possible RF exposure at the workplace. Analyses were adjusted for region, sex, age, SES, urban/rural residence, ionizing radiation exposure in the head/neck region. Mobile phone use was not considered as a confounder.

While overall RF exposure at workplaces showed no increased odds-ratios, high exposure and especially for durations of 10 years or more resulted in elevated risk estimates that were, however, not significant. This result was similar for meningioma (OR=1.55 for high exposure for 10 years or more) and glioma (OR=1.39).

The study tried to assess potential workplace exposure as precisely as possible in a personal interview, but still misclassification may have occurred especially in the probable and not probable categories while the high exposure group is likely to have had at least occasionally above average RF exposure. Odds ratios are in the range expected if exposure results in a substantial increase of growth rate. The small number of highly and long-term exposed cases (13 glioma and 6 meningioma) prohibit, however, far reaching conclusions.

IV. Evaluation of Evidence

Due to the varying endpoints, methods used and populations included and the small number of studies a formal meta-analysis is not possible. The following figure shows the results detailed in Table 2 in an easily comprehendible way.

Only few studies found clear indications of an association between RF exposure and brain tumors: one cohort study (Szmigielski 1996) and two case-control studies (Thomas et al. 1987, Grayson 1996). None of the ecological studies demonstrated a tendency for an increased risk in the vicinity of RF transmitters.

The discussion of the 15 published investigations revealed shortcomings in all studies. The greatest problem was encountered in the difficulties to reliably assess actual exposure. Even if we don't know the relevant aspect of the exposure, if any, that is responsible for an increased risk, the type, duration and amount of exposure must be determined in order to use the studies in derivations of exposure standards. None of the studies included a useful quantitative indicator of intensity of exposure and even duration of exposure was rarely addressed. Concerning type of exposure only quite crude and broad categories were used.

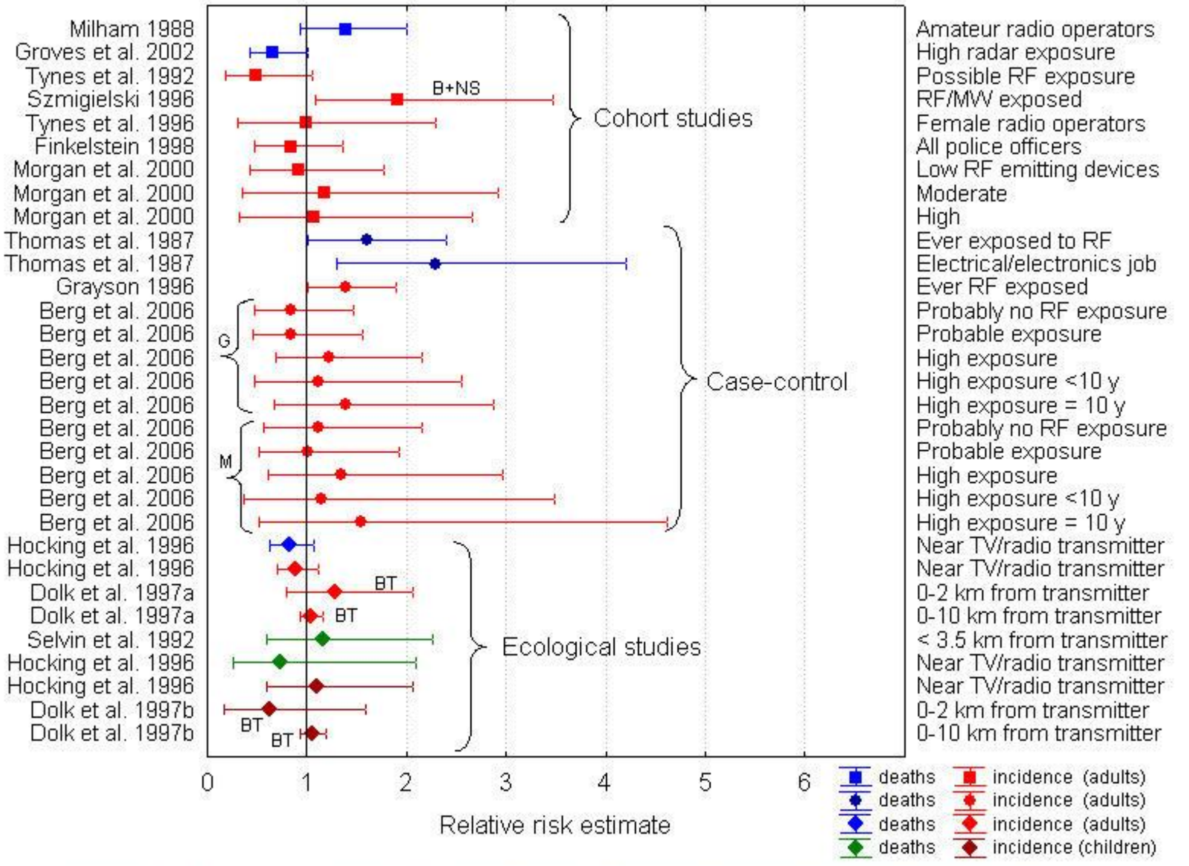

Fig. 1: Estimates of relative risk (and 95% confidence intervals) of various RF exposures with respect to brain tumors (B+NS…brain and nervous system tumors, BT…brain tumors, M…menigioma, G…glioma; all others primary malignant brain tumors)

In ecological studies, although for the studied population the exposure - despite considerable variations in time - is similar with respect to carrier frequency, modulation etc. it is quite different between various types of transmitters and hence results are not easily generalized.
Considering the discussion of the different investigations and the fact that most biases encountered tend to dilute a potential risk, the compiled evidence from occupational cohorts is compatible with a moderately increased risk of RF exposure. Because of the lack of actual measurements but observing that exposure above guideline levels must have been a rare event a precautionary approach must result in a reduction of occupational exposure levels and organizational measures to avoid over-exposure. Although brain tumors are rare and the population attributable risk is low (assuming 13% of adults being occupationally exposed to RF fields as inferred from Berg et al. 2006, and assuming a relative risk of 1.3, about 4% of brain tumors can be attributed to RF exposure, i.e. 1,350 cases per years in the US).

V. EVALUATION OF CANCER-RELATED ENDPOINTS (RF EXPOSURE)

A. Assessment of Epidemiological Evidence by IEEE (C95.1 Revision)

In their 2006 revision of the standard C95.1 IEEE has assessed the evidence from epidemiology for cancer related endpoints in chapter B.7.3. The assessment relies mainly on the reviews of Bergqvist (1997), Moulder et al. (1999) and Elwood (2003). These reviews and the IEEE overview share the same deficiencies. The main lines of argumentation would be impossible in any other field of environmental health and closely resemble the strategy used to dismiss a power frequency exposure/childhood leukemia association. In the following paragraphs the assessment by IEEE will be briefly discussed.

> *Cluster studies, such as the one performed in Sutton Coldfield in the U.K. in response to a cluster of leukemia and lymphoma in adults living close to an RF broadcasting transmitter (Dolk et al. [R624]), are inherently difficult to interpret because of the impossibility of assessing all of the effects that chance variation might have contributed to the cluster. In the initial Sutton Coldfield study, the authors correctly concluded that no causal association could be drawn between the presence of the cluster and RF exposure from broadcasting towers (Dolk et al. [R625]) (Cooper et al. [R760]). (IEEE C 95.1 – 2005, p.75)*

First of all the Sutton Coldfield study was no cluster study but an ecological investigation. It is true that it was initiated by an unconfirmed report of a cluster of leukemia and lymphoma in the vicinity of a broadcasting transmitter but it proceeded independently of this initial report and used registry data on the population living within a radius of 10 km around the transmitter. The statement that such studies are "inherently difficult to interpret because of the impossibility of assessing all of the effects that chance variation might have contributed to the cluster" is ridiculous not only because the study is no cluster study but because it is impossible for any study to "assess all effects that chance variation might have contributed" to the endpoint under investigation. It is not mentioned that the study was supplemented by a larger investigation of another 20 high-power transmitters in Great Britain. The difficulties of interpreting ecological studies is related to the fact that potential confounders can only be related to a segment of the population but not to individuals and that in general duration and intensity of exposure are not known for individual members of the different strata. While evidence for an effect on brain tumor incidence from both studies (Dolk et al. 1997a, 1997b) is weak, there is consistent evidence for a relation to hematopoietic cancers. This evidence has been overlooked by the authors due their wrong assumption about the relation between proximity to the transmitter and exposure.

> *Inconsistent effects have been reported between residential proximity to other RF broadcast towers and adverse health endpoints (Bielski [R267]) (Maskarinec et al. [R579]) (Selvin and Merrill [R823]) (Michelozzi et al. [R858]) (Altpeter et al. [R977]) (Hallberg and Johansson [R995], [R996]) (Boscolo [R1012]), although many of these studies have significant flaws in their study design (making them difficult to interpret). (IEEE C 95.1 – 2005, p.75)*

Although it is not stated what these "inconsistent effects" might be, the statement is flawed in more than this respect. First of all the study by Bielski (1994) is an occupational investigation and not about residential proximity to RF broadcast towers, second three of these investigations (Selvin et al. 1992; Maskarinec et al. 1994; Michelozzi et al. 2002) included leukemia as an endpoint with indications of an increased incidence consistent with the studies from Great Britain (Dolk et al. 1997a, 1997b) and Australia (Hocking et al. 1996). Note that the study by Selvin et al. (1992), as stated previously, intended to compare different methods to assess the relationship between a point source and diseases and did erroneously assume a monotonous relationship between exposure and distance from a transmitter. Correcting this error there seems to be an increased probability of childhood leukemia in areas receiving the highest exposure from the Sutro tower. The other three investigations (Altpeter et al. 1995; Boscolo 2001; Hallberg & Johansson 2002) have nothing in common and hence cannot be inconsistent.

> *An increased incidence and mortality rate of childhood leukemia was reported in Australia with residential proximity to a specific RF broadcasting tower (Hocking et al. [R633]), although subsequent reanalysis of the data showed the results may have been influenced by other confounding variables within the study location (McKenzie et al. [R669]). (IEEE C 95.1 – 2005, p.75)*

This is another example how carelessly and sloppy the evidence is dealt with by the IEEE committee. The study of Hocking et al. (1996) was not about "proximity to a specific RF broadcasting tower" but about an area where three broadcasting towers are located. While there is always the possibility of confounders influencing results of an epidemiologic investigation, the 'reanalysis' of McKenzie et al. (1998) is seriously flawed and cannot support the cited statement. Hocking et al. (1996) combined the districts near the broadcasting area and those further away based on homogeneity analyses, while McKenzie et al. (1998) omitted one area with high incidence (and highest exposure) based on inspection of data. Any statistical analysis subsequent to such data picking is useless.

> *While scattered reports of adverse health effects associated with occupational exposure to RF do exist (Demers et al. [R36]) (Kurt and Milham [R68]) (Pearce [R110]) (Speers et al. [R125]) (Thomas et al. [R128]) (Pearce et al. [R199], [R211]) (Hayes et al. [R207]) (Cantor et al. [R268]) (Davis and Mostofi [R563]) (Tynes et al. [R570], [R605]) (Grayson [R592]) (Richter et al. [R747]) (Holly et al. [R838]) these studies are largely inconsistent with each other in terms of the adverse health endpoints affected, and often show no clear dose response with RF exposure. Many have serious flaws in their study design, contain limited or insufficient RF exposure assessment, and are generally inconsistent with the absence of findings of an association from other occupational studies (Tornqvist et al. [R131]) (Coleman [R142]) (Lilienfeld et al. [R146]) (Robinette and Silverman [R147], [R148]) (Siekierzynski et al. [R151], [R152]) (Wright et al. [R213]) (Coleman et al. [R214]) (Muhm [R506]) (Czerski et al. [R542]) (Hill [R568]) (Lagorio et al. [R616]) (Kaplan et al. [R647]) (Morgan et al. [R701]) (Gallagher et al. [R822]) (Groves et al. [R853]) (Wiklund [R1013]) (Armstrong et al. [R1014]). (IEEE C 95.1 – 2005, p.75)*

Even allowing for restrictions of space for a discussion of the evidence, greater nonsense has not been produced so far in this field as condensed in these two sentences. Putting higgledy-piggledy all sorts of studies together and then wondering about endpoints being inconsistent is an intellectual masterpiece. Of the occupational studies mentioned, three (Thomas et al. 1987; Speers et al. 1988; Grayson 1996) were about brain cancer, three about hematopoietic cancers (Pearce et al. 1985; Kurt & Milham 1988; Pearce 1988), two about testicular cancer (Hayes et al. 1990; Davis & Mostofi 1993), one about male (Demers et al. 1991) and two about female breast cancer (Cantor et al. 1995, Tynes et al. 1996) the latter including other cancers as well, and one about intraocular melanoma (Holly et al. 1996). Three further studies (Pearce et al. 1989; Tynes et al. 1992; Richter et al. 2000) investigated several or all malignancies. These studies differ not only in endpoints, study type (cohort, case-control, and cluster) but also in the methods of exposure assessment. Ignorance of the IEEE reviewers is underlined by the compilation of studies characterized by an "absence of findings of an association". Not only did several of these studies indeed indicate an association of cancer risk with EMF exposure (Lilienfeld et al. 1978; Robinette et al. 1980; Tornqvist et al. 1991; Armstrong et al. 1994; Lagorio et al. 1997; Groves et al. 2002) but two were no epidemiologic studies at all (Siekierzynski et al. 1974; Czerski et al. 1974) and several were rather addressing ELF exposure (Tornqvist et al. 1991; Wright et al. 1982; Coleman et al. 1983; Gallagher et al. 1991) and one (Wiklund 1981) was a cluster study in the telecommunication administration with uncertain type of exposure. Simply confronting studies finding an effect with others that were 'negative' is scientifically flawed and permits neither the conclusion that there is nor that there is no association between exposure and cancer risk. Even if all studies would have applied the same method, assessed the same endpoint and used the same exposure metric, studies reporting a significantly increased cancer risk are not outweighed by others that did not.

> *While micronuclei formation in workers occupationally exposed from broadcast antennas has been reported (Garaj-Vrhovac [R757]) (Lalic et al. [R791]), these findings were not verified in a larger study of more than 40 Australian linemen exposed under similar conditions (Garson et al. [R186]). (IEEE C 95.1 – 2005, pp.75-76)*

It goes without saying that also this statement is wrong. Garson et al. (1991) did not investigate micronuclei formation, their workers were considerably shorter exposed and it were not more than 40 linemen but 38 radio-lineman.

> *No clear association could be established between occupational exposures of parents to a number of agents, including RF, and effects (neuroblastoma) in their offspring (Spitz and Johnson [R289]) (De Roos et al. [R798]). (IEEE C 95.1 – 2005, p.76)*

What is meant by 'no clear association' is obscure. Spitz and Johnson (1985) found a significantly increased risk for paternal occupational exposure to electromagnetic fields, and also De Roos et al. (2001) found several jobs with paternal as well as maternal exposure to EMFs associated with an elevated risk for neuroblastoma in their children. However, broad groupings of occupations with ELF, RF EMF, as well as ionizing radiation (!) exposure did not reveal an increased risk.

> *One study reported a slight excess in brain tumors associated with combined exposure to RF and other exposures associated with electrical or electronic jobs, but not with RF alone (Thomas et al. [R128]). A study of a Polish military cohort reported a substantial excess of total cancer and several cancer sub-types with jobs associated with RF exposure (Szmigielski [R578]), (Szmigielski and Kubacki [R982]), although questions have been raised about severe bias in the exposure assessment of this study (Elwood [R665]) (Bergqvist [R1015]) (Stewart [R1133]). Studies by Milham of U.S. amateur radio operators reported an excess in one of nine types of leukemia assessed (see [R101], [R102], [R209], [R215], and [R569]), but not for total tumors, total leukemia, or brain tumors, and potential confounding factors might have included exposure to soldering fumes, degreasing agents and over-representation of a particular social class. (IEEE C 95.1 – 2005, p.76)*

Again the evidence is incorrectly summarized for all cited investigations. Thomas et al. (1987) found a significantly elevated risk for brain tumors among all men exposed to RF fields and in particular in those exposed for 20 or more years. There were indications that this elevated risk is due to a subgroup with electrical or electronics jobs. The group of those exposed in other jobs is heterogeneous and may contain subjects with low or no exposure (e.g. some groups of welders) and therefore lack of an association could be due to a dilution effect from exposure misclassification.

As mentioned previously criticism of the Polish military cohort study about exposure assessment is unfounded. Bergqvist (1997), Elwood (1999) and Stewart (2000) criticized that the military health board assessed a number of potential risk factors only for cancer cases. However, they overlooked that the study was a cohort and not a case-control study and that at no stage information about these factors entered the analysis and therefore couldn't affect the results in any way.

The study by Milham (1988a, 1988b) of radio amateur operators revealed a significantly increased standardized mortality ratio (SMR) for acute myeloid leukemia while the overall mortality and cancer mortality was significantly reduced relative to the country mortality rates. As mentioned previously this points to a 'healthy worker' effect as well as to an influence of life-style factors (mortality related to smoking and overweight were reduced). From the mentioned nine types of leukemia three with expectancies below one and no case observed couldn't be assessed, from the six remaining types five had elevated SMRs with AML, the most frequent type in adults, being significantly elevated.

The last portion of the IEEE review of epidemiology studies is dedicated to mobile phone investigations that are discussed in another contribution.

The following citation presents the IEEE summary in its full length:

> *The epidemiological evidence to date does not show clear or consistent evidence to indicate a causal role of RF exposures in connection with human cancer or other disease endpoints. Many of the relevant studies, however, are weak in terms of their design, their lack of detailed exposure assessment, and have potential biases in the data. While the available results do not indicate a strong causal association, they cannot establish the absence of a hazard. They do indicate that for commonly encountered RF exposures, any health effects, if they exist, must be small. Even though epidemiological evidence cannot rule out a causal relationship, the overall weight-of-evidence is consistent with the results of the long term animal studies showing no evidence of physiological, pathological or disease-specific effects. (IEEE C95.1 - 2005; pp.76-77)*

As already pointed out earlier (Kundi 2006) there is an intolerable tendency in the past years that confronted with an undeniable epidemiologic evidence of an association between an agent and adverse health effects such as cancer, interested parties take their resort to the concept of causality based on the wrong assumption evidence to "indicate a causal role" is a lot more difficult to provide. Unprecedented, however, is the notion of "a strong causal association". Whatever the meaning of this exceptional statement, the conclusion that, if health effects of commonly encountered RF exposures exist, they must be small, is wrong. To the contrary: considering the "lack of detailed exposure assessment" and other potential biases that predominantly lead to an underestimation of the risk, the evidence points to a quite substantial hazard. While the animal studies reviewed in another section of the IEEE standard document cannot be discussed here it should be underlined that they are generally insufficient to support either an increased risk or the lack of health relevant effects. Therefore they cannot be used in a weight-of-evidence statement as has been made by IEEE, that there is no evidence for adverse health effects of RF exposure.

VI. CONCLUSIONS

- Only few studies of long-term exposure to low levels of RF fields and brain tumors exist, all of which have methodological shortcomings including lack of quantitative exposure assessment. Given the crude exposure categories and the likelihood of a bias towards the null hypothesis of no association the body of evidence is consistent with a moderately elevated risk.

- Occupational studies indicate that long term exposure at workplaces may be associated with an elevated brain tumor risk.

- Although in some occupations and especially in military jobs current exposure guidelines may have sometimes been reached or exceeded, overall the evidence suggest that long-term exposure to levels generally lying below current guideline levels still carry the risk of increasing the incidence of brain tumors.

- Although the population attributable risk is low (likely below 4%), still more than 1,000 cases per year in the US can be attributed to RF exposure at workplaces alone. Due to the lack of conclusive studies of environmental RF exposure and brain tumors the potential of these exposures to increase the risk cannot be estimated.

- Epidemiological studies as reviewed in the IEEE C95.1 revision (2006) are deficient to the extent that the entire analysis is professionally unsupportable. IEEEs dismissal of epidemiological studies that link RF exposure to cancer endpoints should be disregarded, as well as any IEEE conclusions drawn from this flawed analysis of epidemiological studies.

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 11 - part 3

Brain Tumors And RF Fields

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Table 1: Synopsis of epidemiologic studies of or including brain tumors (1987 – 2006)

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Thomas et al. 1987	Northern New Jersey, Philadelphia, gulf coast of Lousiana/1979-1981/Case-control	Interviews with next-of-kin about occupational history – response rates: cases 74%, controls 63%; JEM (2 methods)	Death certificates verified through review of hospital records	age(m), (only males), year of death(m), area of residence(m), educational level, (lead, soldering fumes)	435/386	Cases: deaths of brain tumor or CNS tumors of white males (age>30) from death certificates Controls: deaths from other causes than brain tumors, epilepsy, etc.
Milham 1988	Washington, California/1979-1984/Cohort	Amateur radio operator license within 1/1979 to 6/1984	Mortality records	age, (only males), race, year of death	29	67829 operators, search of deaths in state registry through 1984
Selvin et al. 1992	San Francisco/1973-1988/Spatial cluster	Distance of center of census tract to microwave tower (Sutro tower)	SEER records	-	35	Search of cancer deaths of white individuals (age<21)
Tynes et al. 1992	Norway/1961-1985 /Occupational cohort	Job title in 1960 and 1970 censuses and expert categorization	Cancer registry	age, (only males)	119 overall, 6 in subgroup with possible RF exposure	Cohort of 37945 male workers identified that had jobs in 1960 with possible EMF exposure. among these 3017 with possible RF exposure
Grayson 1996	US Air Force/1970-	Detailed job	Screening of	age(m),	230/920	Cohort of ~880000

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
	1989/Nested case-control	history and classification based on JEM (RF/MW exposure from frequent measurements)	hospital discharge records	race(m), military rank, (ELF and ionizing radiation exposure)		US Air Force members with at least one completed year of service within the study period, no follow up after subjects left service
Szmigielski 1996	Poland (military)/1971-1985/Occupational cohort	Allocation to RF/MW exposure group based on service records, documented measurements of military safety groups	Incident cases from central and regional military hospitals and military health departments	age, (only males)	~46	Annual number of ~127800 military career personnel, ~3720 RF/MW exposed per year
Hocking et al. 1996	Sydney (Australia)/ 1972-1990/Ecological	Municipalities within ~4 km of 3 TV broadcasting towers considered higher exposed as compared to 6 further away	Incident and death cases from cancer registry	age, sex, calendar period	740 (incident) 606 (mortality) 64 age<15 (incident) 30 age<15 (mortality)	Study population: inner area ~135000, outer area ~450000
Tynes et al. 1996	Norway/1961-1991/ Occupational cohort	Certified radio and telegraph	Cancer registry	age, (only females)	5	2619 women certified as radio or telegraph

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
		operators 1920-1980 (98% worked on merchant ships); spot measurements on ships with old-fashioned equipment				operators by Norwegian Telecom
Dolk et al. 1997a	Birmingham (GB)/ 1974-1986/Ecological	Living near a TV/FM radio transmitter (Sutton Coldfield)	Cancer registry	age, sex, calendar year, SES	332	Population (age≥15) ~408000 within 10 km of the transmitter
Dolk et al. 1997b	GB/1974-1986/ Ecological	Living near a high power (≥500 kW erp) transmitter (overall 21)	Cancer registry	age, sex, calendar year, SES	244	Population (age<15) within 10 km of one of 20 high power transmitters
Lagorio et al. 1997	Italy/1962-1992/ Occupational cohort	Working as RF heat-sealer operator	Cancer deaths from registry	age, (only females), calendar period, region	1	302 women employed 1962-1992 in a plastic-ware manufacturing plant as RF sealers
Finkelstein 1998	Ontario (Canada)/ 1964-1995/ Occupational cohort	Working as a police officer (possible	Cancer registry	age, (only males), calendar year	16	20601 male officers of Ontario Police

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
		handheld radar exposure)				
Morgan et al. 2000	USA/1976-1996/ Occupational cohort	Jobs classified according to work with RF emitting devices with different output power	Death certificates from states' statistics offices	age, sex, period of hire	51	All U.S. Motorola employees with at least 1 day employment 1976-1996 (195775 workers, 2,7 million person-years)
Groves et al. 2002	USA/1950-1997/ Occupational cohort	6 occupational groups 3 with assumed low radar exposure (radar-, radio operator, aviation electrician's mate) and 3 with assumed high exposure (aviation electronics -, electronics -, fire control technician)	Death certificate from a state vital statistics office or National Death Index Plus	age at entry, (only males), attained age	88	40581 Navy Korean War veterans graduated 1950-54 from Navy technical schools; follow-up from graduation through 1997
Berg et al. 2006	Germany/2000-2003/ Case-control	JEM from occupational history collected in interview	Histological verified cases of glioma and meningioma	age(m), sex(m), region(m), SES, urban/rural, smoking,	Glioma 366/732 Meningioma 381/762	All histological confirmed cases of glioma and meningioma from 4

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
				ionizing rad. exposure		neurosurgical clinics (age: 30-69) (part.rate 84%); frequency matched controls from population registry (part.rate 63%)

SES…socio-economic status, JEM…job exposure matrix, erp…equivalent radiation power, RF/MW…radio frequency/microwaves, CNS…central nervous system, ELF…extremely low frequency

Table 2: Synopsis of main results of brain tumor studies (1987 – 2006)

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
Thomas et al. 1987	Brain tumor deaths (ICD not specified)	Ever exposed to RF	OR	1.6 [1.0 – 2.4]
		Electrical/electronics job	OR	2.3 [1.3 – 4.2]
		Unexposed*		
		Ever exposed < 5 y	OR	1.0
		5-19 y	OR	2.3
		20+ y	OR	2.0
Milham 1988	Brain cancer deaths (ICD-8: 191)	All	SMR	1.39 [0.93 – 2.00]
		Novice[a]	SMR	0.34
		Technician	SMR	1.12
		General	SMR	1.75
		Advanced	SMR	1.74
		Extra	SMR	1.14
Selvin et al. 1992	Brain cancer deaths (ICD-O: 191.2)	> 3.5 km distance from tower*		
		≤ 3.5 km[b]	RR	1.16 [0.60 – 2.26]
Tynes et al. 1992	Incident brain cancer (ICD-7: 193)	All with possible EMF exposure	SIR	1.09 [0.90 – 1.41]
		Subgroup possible RF exposure[c]	SIR	0.49 [0.18 – 1.06]
Grayson 1996	Incident brain cancer (ICD-9: 191)	Never RF/MW exposed*		
		Ever exposed	OR	1.39 [1.01 – 1.90]
Szmigielski 1996	Incident nervous system & brain tumors	RF/MW exposed	OER	1.91 [1.08 – 3.47]
Hocking et al. 1996	Brain cancer (ICD-9: 191)	Outer area*		
		Inner area (incident, overall)	RR	0.89 [0.71 – 1.11]
		Inner area (mortality, overall)	RR	0.82 [0.63 – 1.07]
		Inner area (incident, age<15)	RR	1.10 [0.59 – 2.06]
		Inner area (mortality, age<15)	RR	0.73 [0.26 – 2.10]
Tynes et al. 1996	Incident brain cancer (ICD-7: 193)	All	SIR	1.0 [0.3 – 2.3]
Dolk et al. 1997a	Incident brain tumors (ICD-8/9: 191, 192)	0-2 km from transmitter	OER	1.29 [0.80 – 2.06]
		0-10 km from transmitter	OER	1.04 [0.94 – 1.16]

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
Dolk et al. 1997b	Incident brain tumors (ICD-8/9: 191, 192)	0-2 km from transmitter	OER	0.62 [0.17 – 1.59]
		0-10 km from transmitter	OER	1.06 [0.93 – 1.20]
Lagorio et al. 1997	Brain cancer deaths (ICD-9: 191)	RF sealer operator	OER	1 : 0.1
Finkelstein 1998	Incident brain cancer (ICD-9: 191)	All police officers	SIR	0.84 [0.48 – 1.36]
Morgan et al. 2000	Incident brain cancer (ICD-9: 191)	No RF exposure*		
		Low[d]	RR	0.92 [0.43 – 1.77]
		Moderate	RR	1.18 [0.36 – 2.92]
		High	RR	1.07 [0.32 – 2.66]
Groves et al. 2002	Brain cancer deaths (ICD-9: 191)	Low radar exposure*		
		High radar exposure	RR	0.65 [0.43 – 1.01]
Berg et al. 2006	Incident glioma (ICD-O3: C71)	No occup. RF/MW exposure*		
		Probably no exposure	OR	0.84 [0.48 – 1.46]
		Probable exposure	OR	0.84 [0.46 – 1.56]
		High exposure	OR	1.22 [0.69 – 2.15]
		No high exposure*		
		High exposure <10 y	OR	1.11 [0.48 – 2.56]
		High exposure ≥ 10 y	OR	1.39 [0.67 – 2.88]
	Incident meningioma (ICD-O3: C70.0)	No occup. RF/MW exposure*		
		Probably no exposure	OR	1.11 [0.57 – 2.15]
		Probable exposure	OR	1.01 [0.52 – 1.93]
		High exposure	OR	1.34 [0.61 – 2.96]
		No high exposure*		
		High exposure <10 y	OR	1.15 [0.37 – 3.48]
		High exposure ≥ 10 y	OR	1.55 [0.52 – 4.62]

[a] From Milham 1988b, license classes as proxy for exposure duration

[b] Based on the assumption that exposure is higher near the microwave tower

[c] Computed based on Table 5 in Tynes et al. 1992

[d] Classification according to power output of equipment used for longest period of employment

OR…odds-ratio, SIR…standardized incidence ratio, SMR…standardized mortality ratio, RR…relative risk (rate ratio), OER…observed/expected ratio

SECTION 11

Use of Wireless Phones and Evidence for Increased Risk of Brain Tumors

2012 Supplement

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I. INTRODUCTION

In May 2011 the International Agency for Research on Cancer (IARC) at WHO categorised the radiofrequency electromagnetic fields (RF-EMF) from mobile phones, and from other devices that emit similar non-ionising electromagnetic fields, as a Group 2B, i.e. a ‘possible’, human carcinogen (Baan et al., 2011, IARC, 2011). Nine years earlier IARC had also classified extremely low frequency (ELF) magnetic field as Group 2B carcinogen (IARC, 2002).

The IARC decision on mobile phones was based mainly on case-control studies from the Hardell group in Sweden and the IARC Interphone study. Both provided supportive results on positive associations between two types of brain tumors; glioma and acoustic neuroma, and exposure to RF-EMF from wireless phones.

The final IARC decision was confirmed by voting of 29 scientists (one not present during voting) at the meeting. A large majority of participants voted to classify RF-EMF radiation as ‘possibly carcinogenic’ to humans, Group 2B. The decision was also based on occupational studies. We present in this paper an updated review of evidence of the association between use of wireless phones and brain tumors including also papers published after the IARC evaluation.

The Nordic countries were among the first countries in the world to widely adopt the wireless telecommunications technology. Analogue phones (NMT; Nordic Mobile Telephone System) were introduced in the early 1980s using both 450 and 900 Megahertz (MHz) frequencies. NMT 450 was used in Sweden from 1981-2007, NMT 900 operated during 1986-2000.

The digital system (GSM; Global System for Mobile Communication) using dual band, 900 and 1800 MHz, started to operate in 1991 and dominates now the market. The third generation of mobile phones, 3G or UMTS (Universal Mobile Telecommunication System), using 1 900/2 100 MHz RF fields has been introduced worldwide in recent years, in Sweden in 2003. Currently the fourth generation, 4G (Terrestrial 3G), operating at 800/2600 MHz and Trunked Radio Communication (TETRA 380-400 MHz) are being established in Europe. Nowadays mobile phones are used more than landline phones in Sweden (http://www.pts.se/upload/Rapporter/Tele/2011/sv-telemarknad-halvar-2011-pts-er-2011-21.pdf). Worldwide, an estimate of 5.9 billion mobile phone subscriptions was reported at the end of 2011 by the International Telecommunication Union (ITU; http://www.itu.int/ITU-D/ict/facts/2011/material/ICTFactsFigures2011.pdf).

Desktop cordless phones (DECT) have been used in Sweden since 1988, first using analogue 800-900 MHz RF fields, but since early 1990s using a digital 1900 MHz system. These cordless phones are becoming more common than traditional landlines. They emit RF-EMF radiation similar to that of mobile phones. Thus when human health risks are evaluated it is also necessary to consider the use of cordless phones along with mobile phones.

The real increase in use and exposure to radiation fields from wireless phones (mobile phones and cordless phones) in most countries has occurred since the end of the 1990s. The brain is the main target organ during use of the handheld phone (Cardis et al., 2008). Fear of an increased risk for brain tumors has dominated the debate during the last one or two decades. While RF-EMFs do not have sufficient energy to break chemical bonds like ionising radiation, at least not directly, they can nevertheless have harmful effects on biological tissues. Plausible biological mechanisms for these effects include DNA damage, impairment of DNA repair mechanisms, and epigenetic changes to DNA (see also chapters by H. Lai (Genotoxicity) and I. Belyaev (Physical and Biological Mechanisms).

Primary brain tumors (central nervous system; CNS) constitute of a heterogeneous group of neoplasms of different histological types depending on tissue of origin with different growth patterns, molecular markers, anatomical localisations, and age and gender distributions. The clinical appearance, treatment and prognosis are quite different depending on tumor type.

There are few established risk factors for brain tumors besides ionising radiation (Preston Martin et al., 2006). Higher socio-economic status tends to be related to higher incidence and some rare inherited cancer syndromes account for a small fraction of tumors (Preston Martin et al., 2006). Familial aggregation of glioma has also been reported (Scheurer et al., 2010).

We base this review primarily on the Hardell group papers and the WHO Interphone study (Interphone Study Group, 2010, 2011, Cardis et al., 2011). More discussion of the results and responses, agreements and disagreements of the findings for the Hardell group and Interphone studies can be found in Hardell et al., (2012, 2013).

II. MATERIALS AND METHODS

The PubMed database (www.ncbi.nlm.nih.gov) was used for an up-dated search of published studies in this area using mobile/cellular/cordless telephone and brain tumour/neoplasm/acoustic neuroma/meningioma/glioma as searching terms. Personal knowledge of published studies was also used in order to get as up-to-date review as possible.

III. RESULTS

Brain tumors overall

Exposure to the radiation from the phones is generally higher in the temporal lobe, the part of the brain that is near to the ear (Cardis et al., 2008). For tumors located in the temporal, occipital or temporoparietal lobe areas of the brain an increased risk was found for ipsilateral exposure, that is the telephone was mostly used on the same side of the head as the tumor appeared, yielding OR = 2.42, 95 % CI = 0.97-6.05 (Hardell et al., 2001). This was the first study in the world that indicated an association between use of mobile phones and an increased risk for brain tumors. However, the results were based on low numbers of exposed subjects and different histopathological types of brain tumors so no firm conclusions could be drawn. Furthermore, this first study did not include use of cordless phones, see also Hardell et al., (1999).

Glioma

Glioma is the most common malignant brain tumor and represents about 60 % of all central nervous system tumors. The most common glioma subtype is astrocytoma. Astrocytic tumors are divided in two groups depending on the malignant potential; low-grade (WHO grades I-II) and high-grade (WHO grades III-IV). Low-grade astrocytoma has a relatively favourable prognosis, whereas survival is shorter for patients with high-grade glioma. Glioblastoma multiforme (WHO grade IV) accounts for 60-75 % of all astrocytoma.

The Hardell group in Sweden studied the association between use of mobile and cordless phones and brain tumors diagnosed during 1997-2003. First, cases diagnosed during 1 January 1997 to 30 June 2000 were included (Hardell et al., 2002, 2003). The next study period included 1 July 2000 to 31 December 2003 (Hardell et al., 2005, 2006a). The methods were the same with the same inclusion criteria and an identical questionnaire in both studies.

In short, both men and women aged 20-80 years at the time of diagnosis were included and all were alive at the time of inclusion in the study. They were reported from cancer registries and had all a brain tumor verified by histopathology. The Swedish Population Registry was used for identification of matched controls. In addition to other exposures use of wireless phones was carefully assessed by a self-administered questionnaire supplemented over the phone. The ear that had mostly been used during calls with mobile phone and/or cordless phone was assessed by separate questions. This information was checked during the supplementary phone calls and finally also by a separate letter with good agreement between these three methods.

Use of the wireless phone was defined as ipsilateral (≥ 50 % of the time), or contralateral (< 50 % of the time) in relation to tumor side. The matched control was assigned the same side as the tumor of the respective case. Use of hands free devices was also assessed as well as use in a car with external antenna. Such use was not included in the calculation of cumulative number of hours for life time use. Latency time was defined as the period from the year of first use until diagnosis (corresponding year for the matched control).

Medical records including computer tomography (CT) and/or magnetic resonance imaging (MRI) were used to define tumor localisation in the brain. Further details can be found in the publications.

As a response to a critique from Boice and McLaughlin (2002) that the exclusion of deceased cases was a source of bias in our studies we performed a study on the cases with a malignant brain tumor that had died before inclusion in the case-control studies 1997-2003. These cases represented patients with a poor prognosis, mostly with astrocytoma WHO grade IV (glioblastoma multiforme). Controls were selected from the Death Registry in Sweden. The study encompassed 464 cases and 464 controls that had died from a malignant disease and 463 controls with other causes of death. Exposure was assessed by a questionnaire sent to the next of kin to each deceased case and control. The questionnaire was similar as in previous studies. This investigation confirmed the previous results of an association between use of mobile phones and malignant brain tumors (Hardell et al., 2010).

We have previously published pooled analysis of malignant brain tumors diagnosed during the period 1997-2003 (Hardell et al., 2006b). These results were updated including also results for the deceased cases with malignant brain tumors (Hardell et al., 2011a, Carlberg, Hardell 2012). The results on use of wireless phones were based on 1,251 cases with malignant brain tumor (response rate 85%) and 2,438 controls (response rate 84%). Most cases had glioma (n=1,148) so we present in the following results for that type of tumor. Latency was divided in three categories, >1-5 years, >5-10 years, and > 10 years from first use of a wireless phone until diagnosis of glioma.

Both use of mobile and cordless phone gave an increased risk overall, highest in the latency group >10 years, increasing further for ipsilateral use yielding for mobile phone OR = 2.9, 95 % CI = 1.8-4.7 and for cordless phone OR = 3.8, 95 % CI = 1.8-8.1 (Table 1). Highest ORs were found in the > 10 year latency group for total wireless phone use as well, OR = 2.1, 95 % CI = 1.6-2.8.
OR increased statistically significant for glioma for cumulative use of wireless phones per 100 h; OR = 1.014, 95 % CI = 1.008-1.019, and per year of latency; OR = 1.056, 95 % CI = 1.037-1.075 (Carlberg and Hardell, 2012). Separate calculations of mobile phone and cordless phone use yielded similar results with statistically significant increasing risks.

The Interphone study was conducted at 16 research centres in 13 countries during varying time periods between 2000 and 2004 under the guidance of IARC. An increased risk for brain tumor was found in some separate country studies and decreased risk in other studies as we have discussed elsewhere (Hardell et al., 2008, 2009). After several years of delay the overall Interphone results were finally published in May 2010 (Interphone Study Group, 2010).

In total 4,301 glioma cases were included in Interphone and the final results were based on 2,708 participating cases (response rate 64 %, range by centre 36-92 %). In total 14,354 potential controls were identified and interviews were completed with 7,658 (53 %, range 42-74 %). The low participation rates in some centres may have created selection bias, see Hardell et al., (2008).

Regular use of mobile phone in the past ≥ 1 year gave for glioma OR = 0.81, 95 % CI = 0.70-0.94 (Table 1). Subgroup analyses showed statistically significant increased risk in the highest exposure group, i.e. those with cumulative mobile phone use ≥ 1,640 hours, OR = 1.40, 95 % CI = 1.03-1.89. The risk increased further for glioma in the temporal lobe yielding OR = 1.87, 95 % CI = 1.09-3.22. In the same exposure category, cumulative use ≥ 1,640 hours and ipsilateral exposure produced OR = 1.96, 95 % CI = 1.22-3.16 in total (no data given for temporal lobe).

In Appendix 2 (Interphone Study Group, 2010, available on the web) analysis was restricted to ever-regular users of mobile phones. Cumulative call time ≥ 1,640 hours gave OR = 1.82, 95 % CI = 1.15-2.89 compared with use < 5 hours. Time since start of regular use (latency) ≥ 10 years produced OR = 2.18, 95 % CI = 1.43-3.31; reference entity 1-1.9 years.

The Interphone study group concluded: "*However, biases and errors limit the strength of the conclusions we can draw from these analyses and prevent a causal interpretation.*" In an editorial accompanying the Interphone results the main conclusion of the Interphone results was described as "*both elegant and oracular…(which) tolerates diametrically opposite readings*" (Saracci and Samet 2010). Several methodological reasons why the Interphone results were likely to have underestimated the risks were discussed including the short latency period since first exposures became widespread; less than 10 % of the Interphone cases had more than 10 years exposure. "*None of the today's established carcinogens, including tobacco, could have been firmly identified as increasing risk in the first 10 years or so since first exposure*".

Estimated RF-EMF dose in the tumor area from mobile phone use was associated with an increased risk of glioma in parts of the Interphone study (Cardis et al., 2011). OR increased with increasing total cumulative dose of specific energy (J/kg) absorbed at the estimated tumor centre for more than 7 years before diagnosis giving OR = 1.91, 95 % CI = 1.05-3.47 (p trend = 0.01) in the highest quintile of exposure. A similar study based on less clear methods was later published by another part of the Interphone study group (Larjavaara et al., 2011). The results seemed not to support the findings of Cardis et al., (2011). However, only 42 cases had used a mobile phone for more than 10 years and no analysis was made of the most exposed group with longest duration of use.

Based on Hardell et al (2011b) and Interphone Study Group (2010) we made meta-analysis of glioma and use of mobile phones. Random-effects model was used based on test for heterogeneity in the overall (≥10 years and ≥1,640 hours) groups. We used published results in

Interphone since we do not have access to their database. Our results were recalculated to these groups of exposure. The meta-analysis yielded for mobile phone use OR = 1.71, 95 % CI = 1.04-2.81 for glioma in the temporal lobe in the ≥ 10 years latency group. Ipsilateral mobile phone use ≥ 1,640 h in total gave the highest risk, OR = 2.29, 95 % CI = 1.56-3.37 (Hardell et al 2012). This meta-analysis strengthens a causal association between use of mobile phones and glioma.

Meningioma

Meningioma is the most common benign brain tumor. It develops from the pia and arachnoid that covers the central nervous system. Meningioma is an encapsulated and well-demarked tumor more common in women than in men. It is rarely malignant.

A pooled analysis of benign brain tumors from the two case-control studies from the Hardell group as discussed above (Hardell et at., 2006c, Hardell and Carlberg, 2009) gave regarding meningioma and use of mobile phone OR = 1.1, 95 % CI = 0.9-1.3, and cordless phone OR = 1.1, 95 % CI = 0.9-1.4 (Table 2). Using > 10 year latency period OR increased; for mobile phone to OR = 1.5, 95 % CI = 0.98-2.4, and for cordless phone to OR = 1.8, 95 % CI = 1.01-3.2. Ipsilateral mobile phone use in the > 10 years latency group yielded OR = 1.6, 95 % CI = 0.9-2.9, and cordless phone OR = 3.0, 95 % CI = 1.3-7.2. These results were based on rather low numbers of exposed cases, however.

Regular use of mobile phone produced in the Interphone study (2010) a statistically significant decreased risk for meningioma, OR = 0.79, 95 % CI = 0.68-0.91, Table 2. The risk increased somewhat with cumulative use ≥ 1,640 hours and ipsilateral mobile phone use to OR = 1.45, 95 % CI = 0.80-2.61. Analysis restricted to tumors in the temporal lobe or to the group of ever-regular use did not change the overall pattern of no increased risk.

We performed meta-analysis of meningioma for use of mobile phone based on results in the Hardell group and Interphone results similarly as for glioma. No statistically significant decreased or increased risk was found (Hardell et al., 2012). These results support the conclusion that up to latency ≥ 10 years or cumulative use ≥1,640 hours there is no consistent pattern of an association between use of mobile phones and meningioma.

Acoustic neuroma

Acoustic neuroma or Vestibular Schwannoma is a slow growing benign tumor located in the eighth cranial nerve in the auditory canal. It grows gradually out into the cerebellopontine angle with potential compression of vital brain stem centres. Tinnitus and hearing problems are usual first symptoms of acoustic neuroma. The eighth cranial nerve is located close to the handheld wireless phone when used, so there is particular concern of an increased risk for neuroma development due to exposure to EMF-RF emissions during use of these devices.

The pooled analysis of the Hardell group studies yielded regarding use of mobile phones for acoustic neuroma OR = 1.7, 95 % CI = 1.2-2.3 increasing to OR = 2.9, 95 % CI = 1.6-5.5 with > 10 years latency period, Table 3. Ipsilateral use increased the risk further; in the > 10 years latency group to OR = 3.0, 95 % CI = 1.4-4.2 (Hardell and Carlberg, 2009). Cordless phone use gave OR = 1.5, 95 % CI = 1.04-2.0 increasing to OR = 1.7, 95 % CI =1.2-2.5 for ipsilateral use in the > 1 year latency group.

In the Interphone study (2011) 1,121 (82 %) acoustic neuroma cases participated, range 70-100 % by centre. Of the controls 7,658 (53 %) completed the interviews, range 35-74 % by centre. The final matched analysis (1:1 or 1:2) consisted of 1,105 cases and 2,145 controls. Overall no increased risk was found censoring exposure at one year or at 5 years before reference date, OR = 0.85, 95 % CI = 0.69-1.04 and OR = 0.95, 95 % CI = 0.77-1.17, respectively (Table 3).

Cumulative number of hours of ipsilateral mobile phone use ≥ 1,640 hours up to 1 year before reference date gave OR = 2.33, 95 % CI = 1.23-4.40 and contralateral use OR = 0.72, 95 % CI = 0.34-1.53 for acoustic neuroma, see Table 3 (Interphone Study Group, 2011). For cumulative number of hours of ipsilateral mobile phone use ≥ 1,640 hours up to 5 years before reference date OR = 3.53, 95 % CI = 1.59-7.82, and for contralateral use OR = 1.69, 95 % CI = 0.43-6.69 were obtained. The risk increased further for cumulative ipsilateral use ≥ 1,640 hours with start ≥ 10 years before reference date to OR = 3.74, 95 % CI = 1.58-8.83. Contralateral use in that group yielded OR = 0.48, 95 % CI = 0.12-1.94, however based on only 4 exposed cases and 9 exposed controls. Overall OR = 1.93, 95 % CI = 1.10-3.38 was obtained for long-term use with start ≥ 10 years before reference date and cumulative call time ≥ 1,640 hours.

Similar analyses of the data as in Appendix 2 for glioma (see Interphone Study Group, 2010) yielded highest OR for acoustic neuroma in the shortest latency group, 2-4 years before reference date, OR = 1.41, 95 % CI = 0.82-2.40. Lower OR was calculated in the ≥ 10 years group, OR = 1.08, 95 % CI = 0.58-2.04. Somewhat higher risk than in total, OR = 1.32, 95 % CI = 0.88-1.97, was found for cumulative mobile phone use ≥ 1,640 hours; OR = 1.74, 95 % CI = 0.90-3.36, in this analysis restricted to only regular users. No results were given for ipsilateral use.

We performed meta-analysis of the results for use of mobile phone and the association with acoustic neuroma based on results by the Hardell group and Interphone study (Hardell et al 2012). For the latency group ≥ 10 years highest risk was obtained for ipsilateral use, OR = 1.81, 95 % CI = 0.73-4.45. The risk increased further for cumulative use ≥ 1,640 hours yielding OR = 2.55, 95 % CI = 1.50-4.40 for ipsilateral use. The meta-analysis strengthens a causal association between use of mobile phones and acoustic neuroma.

A case-case study was performed in Japan (Sato et al., 2011). The cases were identified during 2000-2006 at 22 participating neurosurgery departments. The diagnosis was based on histopathology or CT/MRI imaging. Of 1,589 cases 816 (51 %) agreed to participate and answered a mailed questionnaire. The final analysis included 787 cases, Cases with ipsilateral use were regarded as exposed and those with contralateral use were assumed to be unexposed and were used as the reference category. Overall no increased risk was found. However, for average daily call duration > 20 minutes with reference date 1 year Risk Ratio (RR) = 2.74, 95 % CI = 1.18-7.85 was found increasing to OR = 3.08, 95 % CI = 1.47-7.41 with reference date 5 years before diagnosis (Table 3). Unfortunately no results were given for cumulative number of hours for use over the years. For cordless phones no increased risk was found but the analysis was not very informative.

Risks to children and adolescents

The developing brain is more sensitive to toxins (Kheifets et al., 2005) and it is still developing until about 20 years of age (Dosenbach et al., 2010). Children have smaller head and thinner skull bone than adults. Their brain tissue has also higher conductivity and these circumstances give higher absorption from RF-EMF than for adults (Cardis et al., 2008, Christ et al., 2010, Gandhi et al., 2012). Use of wireless phones is widespread among children and adolescents

(Söderqvist et al., 2007, 2008). The greater absorption of RF energy per unit of time, the greater sensitivity of their brains, and their longer lifetimes with the risk to develop a brain tumor leaves children at a higher risk than adults from mobile phone radiation.

We have published results regarding brain tumor risk for different age groups at the time of diagnosis (Hardell et al., 2004) or age at first use of wireless phones (Hardell and Carlberg, 2009, Hardell et al., 2011a, 2012, 2013). Three age groups for first use of a wireless phone were used: <20 years, 20-49 years and 50-80 years. Highest risk for glioma was found for first use of mobile phone or cordless phone before the age of 20 years (Table 4). Thus, mobile phone use yielded for glioma OR = 3.1, 95 % CI = 1.4-6.7 and cordless phone OR 2.6, 95 % CI = 1.2-5.5.

Also for acoustic neuroma the risk was highest in the youngest age group with OR = 5.0, 95 % CI = 1.5-16 for use of mobile phone. Only one case had first use of cordless phone before the age of 20, so no conclusions could be drawn for cordless phones. Regarding meningioma no clear pattern of age-dependent increased risk was seen.

A multi-centre case-control study was conducted in Denmark, Sweden, Norway, and Switzerland, CEFALO (Aydin et al., 2011). It included children and adolescents aged 7–19 years and has been commented elsewhere in detail since serious methodological problems exist in the study design and interpretation of the results (Söderqvist et al., 2011). In CEFALO a statistically non-significant increased risk for brain tumors among regular users (one call per week for at least 6 months) of mobile phones was found; OR = 1.36, 95 % CI = 0.92-2.02. This OR increased somewhat with cumulative duration of subscriptions and duration of calls (Aydin et al., 2011). No data for long-term use were given; the longest latency period was 5 years. Further support of a true association was found in the results based on operator-recorded use for 62 cases and 101 controls, which for time since first subscription >2.8 years yielded a statistically significant OR of 2.15, 95 % CI = 1.07-4.29, with a statistically significant trend (p=0.001).

Use of cordless phones was covered only in the first 3 years of use. No explanation was given for this most peculiar definition. Wireless phone use was not considered, that is use of both mobile phones and cordless phones as the relevant exposure category, as used by the Hardell group and adopted by IARC (Baan et al., 2011). Instead Aydin et al., (2011) included use of cordless phones in the ‘unexposed’ category when risk estimates were calculated for mobile phone use. Similarly, regarding use of cordless phones RF-EMF emissions from mobile phones were regarded as ‘no exposure’. Thus, an increased risk was potentially concealed.

The authors summarised that they “*did not observe that regular use of a mobile phone increased the risk for brain tumors*.” An editorial in the same journal accompanied that conclusion by stating by that the study showed “*no increased risk of brain tumors*” (Boice and Tarone, 2011). This was echoed by a news release from the Karolinska Institute in Stockholm claiming that the results of no increased risk were ‘reassuring’ (Karolinska Institute, 2011). However the results indicate a moderately increased risk, in spite of low exposure, short latency period and limitations in study design and analyses. Certainly it cannot be used as reassuring evidence against an association, see Söderqvist et al., (2011).

Danish cohort study on mobile phone subscribers

An attempt to establish a cohort of mobile phone users was made in Denmark in co-operation between the Danish Cancer Society and the International Epidemiology Institute (IEI), Rockville, MD, USA. It was financed by grants from two Danish telecom operation companies (TeleDenmark Mobil and Sonafon), IEI, and the Danish Cancer Society. The source of money for IEI has not been disclosed.

The Danish study on brain tumor risk among mobile phone subscribers has so far resulted in four publications (Johansen et al., 2001, Schüz et al., 2006, Frei et al., 2011, Schüz et al., 2011). It included subjects from January 1, 1982 until December 31, 1995 identified from the computerised files of the two Danish operating companies, TeleDenmark Mobil and Sonofon. A total of 723,421 subscribers were initially identified but the final cohort consisted of only 58 % of these subjects. Due to lack of names of individual users 200,507 corporate users were excluded.

We have discussed elsewhere several shortcomings in the Danish cohort study such as exclusion of corporate users, no individual exposure data, users of cordless phones are included in the reference category, no control for use of mobile phones in the population after the establishment of the cohort, and no operator-verified data on years of subscription is available (Söderqvist et al., 2012). These limitations are likely to have led to an underestimate of any risk in this study.

One would also expect considerable misclassification of mobile phone use both among subscribers and the reference population since no new subscribers were included in the exposed cohort after 1995.

The IARC working group concluded that the methods used could have resulted in considerable misclassification in exposure assessment in the Danish cohort study on mobile phone subscribers (Baan et al., 2011).

After the outcome of the IARC-evaluation was made public in June 2011 (Baan et al., 2011) two additional reports on the Danish cohort were published (Frei et al., 2011, Schüz et al., 2011). Both were new up-dates of the initial cohort and included more information on risk related to longer follow-up. One focused on acoustic neuroma (Schüz et al., 2011) while the other gave results both for all cancers and separately for glioma and meningioma (Frei et al., 2011). This time the number of the cohort was reduced to 358,403 (49.5 %) of the initially identified subscribers (n=723,421). The major additional exclusion (n=54,350) was due to record linkage with the Danish so-called CANULI cohort on socioeconomic factors (Dalton et al., 2008).

The authors of the Danish study have themselves pointed out the main causes of considerable exposure misclassifications (Frei et al., 2011). While at least non-response and recall bias can be excluded the study has serious limitations related to exposure assessment (Söderqvist et al., 2012). In fact, these limitations cloud the findings of the four reports to such an extent they are uninformative at best. At worst, they may be used in a seemingly solid argument against an increased risk; as reassuring results from a large nationwide cohort study.

Brain tumor incidence

It has been suggested that overall incidence data on brain tumors for countries show no increasing trends and may be used to disqualify the association between mobile phone use and brain tumors observed in the case-control studies (Aydin et al., 2011; Ahlbom, and Feychting, 2011; Deltour et al., 2012; Little et al., 2012).

However, by now several studies show increasing incidence of brain tumors. In Denmark a statistically significant increase in incidence rate per year for brain and central nervous system tumors (combined) was seen during 2000-2009; in men +2.7 %, 95 % CI = +1.1 to 4.3 % and in women +2.9 %, 95 % CI = +0.7 to 5.2 % (NORDCAN). Updated results for brain and central nervous system tumors have been released in Denmark. The age-standardized incidence of brain and central nervous system tumors increased with 40 % among men and 29 % among women during 2001-2010 (Sundhedsstyrelsen, 2010). A more recent news release based on the Danish Cancer Register stated that during the last 10 years there has been an increasing number of cases with the most malignant glioma type, glioblastoma multiforme (astrocytoma WHO grade IV), especially among men (http://www.cancer.dk/Nyheder/nyhedsartikler/2012kv4/Kraftig+stigning+i+hjernesvulster.htm).

Little et al., (2012) studied the incidence rates of glioma during 1992-2008 in the United States and compared with ORs for glioma associated with mobile phone use in the 2010 Interphone publication (Interphone Study Group, 2010) and our pooled results published in 2011 (Hardell et al., 2011a). Since our results are discussed and questioned by Little et al their study needs to be reviewed in more detail. Our response to the journal (BMJ) was never accepted for publication in the journal and cannot be found via PubMed, only on the web (http://www.bmj.com/content/344/bmj.e1147/rr/578564).

First, one important methodological issue that was not stated in the abstract or in the article [Figures 2-4] by Little et al., (2012), but can be found in the web appendix, is that observed rates were based on men aged 60-64 years from the Los Angeles SEER registry as the baseline category. These data were used to estimate rates in the entire dataset, men and women aged ≥ 18 years and all 12 SEER registries. Thereby numerous assumptions were made as pointed out by Kundi (2012) and Davis et al., (2012).

Using only men, as Little et al., did, ignores the fact that women had less frequent use of mobile phones than men in our studies (Table 5). Overall 31 % of women reported such use *versus* 57 % of men. Furthermore, use varies with age group with a large difference according to age, as we have explored in our publications (Hardell and Carlberg, 2009, Hardell et al., 2011a). Thus, the age group 60-64 year old men is not valid to use for these calculations.

There are several other points that may be added. Another example is that the results for anatomical localisations and tumor grade [in Table 5 in the article] by Little et al are based on numerous assumptions from SEER data, Interphone and the Hardell group studies. The authors seem not to have paid attention to the fact that the fraction of mobile phone users differs for gender and age, see Table 5.

One interesting result that was not commented further by Little et al., (2012) was the finding of a statistically significant yearly increasing incidence of high-grade glioma (WHO grades III-IV) in the SEER data for 1992-2008, +0.64%, 95% CI = +0.33 to 0.95 %. On the contrary, the incidence of low-grade glioma (WHO grades I-II) decreased with –3.02 %, 95 % CI = –3.49 to –2.54 %. Little et al., (2012) found also a statistically significant increasing yearly trend for glioma in the temporal lobe, +0.73 %, 95 % CI = +0.23 to 1.23 %.

Zada et al., (2012) studied incidence trends of primary malignant brain tumors in the Los Angeles area during 1992-2006. The overall incidence of primary malignant brain tumors decreased over the time period with the exception of glioblastoma multiforme (astrocytoma WHO grade IV). The annual age adjusted incidence rate of that tumor type increased statistically significant in the frontal lobe with Annual Percentage Change (APC) +2.4 % to +3.0 % ($p \leq 0.001$) and temporal lobe APC +1.3 % to +2.3 % ($p \leq 0.027$) across all registries. In the California Cancer Registry the incidence of glioblastoma multiforme increased also in cerebellum, APC +11.9 % ($p < 0.001$). For lower grade astrocytoma decreases of annual age adjusted incidence rates were observed. The authors concluded that there was a real increase in the incidence of glioblastoma multiforme in frontal and temporal lobes and cerebellum, areas of the brain with the highest absorbed dose of RF-EMF emissions from handheld mobile phones (Cardis et al., 2008).

Of interest is also the report by de Vocht et al., (2011) from England that showed for the time period 1998 to 2007 a statistically significant increasing incidence of brain tumors, the majority glioma, in the temporal lobe for men and women ($p < 0.01$), and frontal lobe for men ($p < 0.01$). The incidence increased also for women in the frontal lobe, although not statistically significant ($p = 0.07$). The incidence decreased in other parts of the brain.

Deltour et al., (2012) reported increasing glioma incidence rates in Denmark, Finland, Norway, and Sweden for the time period 1979-2008. APC increased for men with +0.4 %, 95 % CI +0.1 to 0.6 % and for women with +0.3 %, 95 % CI +0.1 to 0.5 %. A study from Australia for the time period 2000-2008 showed that APC for malignant brain tumors increased statistically significant +3.9 %, 95 % CI +2.4 to 5.4 % (Dobes et al., 2011). An increase was seen among both men and women. The APC for benign tumors increased with +1.7 %, 95 % CI -1.4 to +4.9 %, thus not statistically significant.

From urban Shanghai an increasing incidence of brain and nervous system tumors for the time period 1983-2007 was reported with APC +1.2 %, 95 % CI +0.4 to 1.9 % in males and APC +2.8 %, 95 % CI +2.1 to 3.4 % in females (Ding and Wang, 2011).

We reported increasing incidence of astrocytoma WHO grades I-IV during 1970-2007 in Sweden. In the age group > 19 years the annual change was +2.16 %, 95 % CI +0.25 to 4.10 % during 2000-2007, for further details see Hardell and Carlberg (2009).

IV. DISCUSSION

As pointed out by IARC (Baan et al., 2011) the most comprehensive results on use of wireless phones and the association with brain tumors come from the Hardell group in Sweden and the international Interphone study. Results for latency time of 10 years or more have been published from both study groups.

Both were case-control studies and the cases were recruited during similar time periods, 1997-2003 in the Hardell group and during 2000-2004 in Interphone, with somewhat different years in the varying study regions. There was no overlapping of cases in the Hardell group studies and the Swedish part of Interphone.

The Hardell group included cases aged 20-80 years whereas eligible cases in Interphone were aged 30-59 years at diagnosis. One control subject matched on age, gender and geographical area (region) to each case in the Hardell group studies was drawn from the national population register. In Interphone one control was selected for each case from a ‘locally appropriate population-based sampling frame’. In Germany two controls were selected and the centres used individual matching or frequency matching. Regarding the Interphone study on acoustic neuroma some centres sampled special controls to the cases, other draw controls from the pool of controls in the glioma and meningioma studies, or used a mixture of both methods. In UK general practioners' lists (Hepworth et al 2006) and in Japan random digit dialling were used (Takebayashi et al., 2006, 2008). Certainly the methods used in Interphone may introduce selection bias.

Use of wireless phones and other exposures were carefully assessed by a self-administered questionnaire in the Hardell et al., studies. The information was supplemented over the phone by trained interviewers thereby using a structured protocol. This was done blinded as to case or control status. After the interviews all personal data like names and addresses were removed from the questionnaires so that only an id-number that did not disclose if it was a case or a control was shown. Thus, coding of the data for statistical analysis was performed without personal data of the individual.

On the contrary information on past mobile phone use was collected during face-to-face interviews in Interphone obviously disclosing if it was a case or a control that was interviewed. These interviews were performed by a large number of interviewers at different participating centres. Experienced interviewers were defined as those who conducted at least 20 interviews. In fact, in the sensitivity analysis the risk increased for glioma for cumulative mobile phone use ≥ 1,640 hours from OR = 1.40, 95 % CI 1.03-1.89 to OR = 1.50, 95 % CI = 1.10-2.06 if 'experienced interviewers only' were considered. The higher risk restricting analysis to 'experienced interviewers' in Interphone indicates observational bias during assessment of exposure decreasing the risk.

In the Hardell group studies few persons conducted all interviews of the 1,251 participating cases with malignant brain tumor, 1,254 cases with benign brain tumor, and 2,438 controls (total 4,942; note one case had both a malignant and a benign brain tumor). All interviewers were first educated; they used a defined protocol and gained considerable experience as interviewers. In fact, they were obliged to carry out the interviews extensively to fulfil the quality in data assessment according to the structured protocol. It is obvious that the few interviewers in the Hardell group study must have been much more experienced than the diversity of interviewers in Interphone.

In the personal interviews in Interphone a computer program that guided the interview with questions read by the interviewer from a laptop computer screen was used. The answers were entered directly into the computer by the interviewer. Using computer based face-to-face interviews may be a stressful situation for the patients. In fact patients scored significantly lower than controls due to recalling of words (aphasia), problems with writing and drawing due to paralysis in the Danish part of Interphone (Christensen et al., 2005). Furthermore, it has not been disclosed how the personal interviews were performed in sparsely populated areas, e.g. in the Northern Sweden. Did the interviewers travel long distances for interviews of controls in rural areas or were all controls living in the largest cities thereby creating selection bias?

In the Hardell group studies the response rate was 85 % (n=1,251) for cases with malignant brain tumor, 88 % (n=1,254) for cases with benign brain tumor, and 84 % (n=2,438) for controls (Hardell et al., 2006c, Carlberg and Hardell, 2012). Lower response rates were obtained in Interphone study, 64 %, range by centre 36-92 %, (n=2,765) for glioma cases, 78 %, range 56-92 %, (n=2,425) for meningioma cases, 82 %, range 70-100 % (n=1,121) for acoustic neuroma cases, and 53 %, range 42-74 %, (n=7,658) for controls (Interphone Study Group, 2010; 2011). These low response rates may have created the possibility of considerable selection bias (Hardell et al., 2008). Not responding controls in Interphone tended to be less frequent users of mobile phone than participating controls leading to underestimation of the risk.

The Hardell group studies included subjects aged 20-80 years, versus 30-59 years in Interphone. We have shown that restricting the age group to 30-59 years and considering subjects that used a cordless phone as unexposed in the Hardell group studies reduced the ORs and produced results quite similar to Interphone (Hardell et al., 2011b). Latency time > 10 years for glioma in the temporal lobe yielded OR = 1.40, 95 % CI = 0.70-2.81 in the Hardell group studies and OR = 1.36, 95 % CI = 0.88-2.11 in Interphone (latency ≥ 10 years). Thus, excluding exposure to RF-EMFs from cordless phones as in the Interphone study as well as excluding the younger and older subjects biased the ORs towards unity in Interphone, which likely dilutes the ability to see health risks.

By placing a strong emphasis on incidence data an association between use of wireless phones and brain tumors has been challenged (Swerdlow et al., 2011). The authors considered that if the increased risks seen in case-control studies reflect a causal relationship, there would already be an increase in incidence of brain and central nervous system tumors. As discussed above by now increasing incidence rates, especially for certain brain tumor types and anatomical localisations of relevance, have been reported. The natural history of most glioma from earliest events to clinical manifestation is unknown, but most likely several decades. The exposure duration in most studies is thus incompatible with a tumor initiating effect. If the exposure on the other hand acts as a promoter, this would decrease latency time for already existing tumors, giving a temporary but not a continuous increase in incidence (Kundi, 2010).

The first case in the world on worker's compensation for a brain tumor after long-term use of wireless phones was the ruling 12 October 2012 by the Italian Supreme Court. A previous ruling that the Insurance Body for Work (INAIL) must grant compensation to a businessman who had used wireless phones for 12 years and developed a neurinoma in the brain was affirmed (http://www.applelettrosmog.it/public/news.php?id_news=44 ; www.microwavenews.com). He had used both mobile and cordless phones for five to six hours per day preferably on the same side as the tumour developed. The neurinoma was located in the trigeminal Gasser's ganglion in the brain. This 5th cranial nerve controls facial sensations and muscles. It is the same type of tumour as the acoustic neuroma in the 8th cranial nerve located in the same area of the brain. No further appeal of the Supreme Court decision is possible.

V. CONCLUSIONS

Based on epidemiological studies there is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of mobile phones and cordless phones. The evidence comes mainly from two study centres, the Hardell group in Sweden and the Interphone Study Group. No consistent pattern of an increased risk is seen for meningioma. A systematic bias in the studies that explains the results would also have been the case for meningioma. The different risk pattern for tumor type strengthens the findings regarding glioma and acoustic neuroma. Meta-analyses of the Hardell group and Interphone studies show an increased risk for glioma and acoustic neuroma. Supportive evidence comes also from anatomical localisation of the tumor to the most exposed area of the brain, cumulative exposure in hours and latency time that all add to the biological relevance of an increased risk. In addition risk calculations based on estimated absorbed dose give strength to the findings.

In summary:

- There is reasonable basis to conclude that RF-EMFs are bioactive and have a potential to cause health impacts.
- There is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of wireless phones (mobile phones and cordless phones) mainly based on results from case-control studies from the Hardell group and Interphone Final Study results.
- Epidemiological evidence gives that RF-EMF should be classified as a human carcinogen.
- Based on our own research and review of other evidence the existing FCC/IEE and ICNIRP public safety limits and reference levels are not adequate to protect public health.
- New public health standards and limits are needed.

Authors' contributions

Lennart Hardell was responsible for drafting of the manuscript and Michael Carlberg made all statistical calculations. Michael Carlberg and Kjell Hansson Mild read and gave valuable comments on the manuscript. All authors have read and approved the final version. No conflicts of interest reported. Supported by grants from Cancer- och Allergifonden, Cancerhjälpen, and Örebro University Hospital Cancer Fund.

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Table 1. Summary of studies on the use of wireless phones and glioma risk

Study	Years Study Type	Age	Tumour type	No. of exposed cases	Odds ratio, 95 % confidence interval	Comments
Hardell et al (2006b, 2010, 2011a) Carlberg, Hardell (2012) Sweden	1997-2003 Case-control	20-80 years	Glioma (n=1148)	123	OR **2.5** (1.8-3.3)	>10 year latency, mobile phone
				57	OR **2.9** (1.8-4.7)	>10 year latency, mobile phone, *ipsilateral*, only living
				50	OR **2.6** (1.7-4.1)	>10 year latency, *mobile phone only*
				45	OR **1.7** (1.1-2.6)	>10 year latency, cordless phone
				20	OR **3.8** (1.8-8.1)	>10 year latency, cordless phone, *ipsilateral*, only living
				9	OR 1.2 (0.5-2.9)	>10 year latency, *cordless phone only;* >5-10 year latency OR **1.9** (1.3-2.9; n=55)
				150	OR **2.1** (1.6-2.8)	>10 year latency, wireless phone (mobile and cordless phone)
			Astrocytoma, high grade (n=820)	102	OR **3.0** (2.1-4.2)	>10 year latency, mobile phone
				47	OR **3.9** (2.3-6.6)	>10 year latency, mobile phone, ipsilateral, only living
				37	OR **2.8** (1.7-4.6)	>10 year latency, *mobile phone only*
				36	OR **2.0** (1.2-3.2)	>10 year latency, cordless phone
				15	OR **5.5** (2.3-13)	>10 year latency, cordless phone, ipsilateral, only living
				6	OR 0.9 (0.3-2.6)	>10 year latency, *cordless phone only;* >5-10 year latency OR **2.4** (1.6-3,7; n=44)
				121	OR **2.5** (1.8-3.4)	>10 year latency, wireless phone (mobile and cordless phone)

Table 1. cont.

Study	**Years** **Study Type**	**Age**	**Tumour type**	**No. of exposed cases**	**Odds ratio, 95 % confidence interval**	**Comments**
Interphone Study Group (2010) 13 countries; Australia, Canada, Denmark, Finland, France, UK, Germany, Israel, Italy, Japan, New Zealand, Norway, Sweden	2000-2004, 2-4 years depending on study region. Case-control	30-59 years	Glioma (n=2708)	1666	OR **0.81** (0.70-0.94)	Regular use of mobile phone in the past ≥1 year
				210	OR **1.40** (1.03-1.89)	Cumulative hours mobile phone ≥ 1640 hours
				78	OR **1.87** (1.09-3.22)	Cumulative hours mobile phone ≥ 1640 hours, tumors in *temporal lobe*
				100	OR **1.96** (1.22-3.16)	Cumulative hours mobile phone ≥ 1640 hours, *ipsilateral* mobile phone use
Interphone Study Group (2010) Appendix 2			Glioma (n=1211)	460	OR **1.68** (1.16-2.41)	Restricted to *ever regular use* time since start 2-4 years; 1-1.9 years as reference entity
				468	OR **1.54** (1.06-2.22)	Restricted to *ever regular use* time since start 5-9 years; 1-1.9 years as reference entity
				190	OR **2.18** (1.43-3.31)	Restricted to *ever regular use* time since start 10+ years; 1-1.9 years as reference entity
				160	OR **1.82** (1.15-2.89)	Restricted to *ever regular use* ≥1640 hours, <5 hours as reference entity

Table 2. Summary of studies on the use of wireless phones and meningioma risk

Study	**Years Study Type**	**Age**	**Tumour type**	**No. of exposed cases**	**Odds ratio, 95 % confidence interval**	**Comments**
Hardell et al (2006c), Hardell, Carlberg (2009) Sweden	1997-2003 Case-control	20-80 years	Meningioma (n=916)	347	OR 1.1 (0.9-1.3)	> 1 year latency, mobile phone use
				38	OR 1.5 (0.98-2.4)	> 10 years latency of mobile phone use
				18	OR 1.6 (0.9-2.9)	> 10 years latency of ipsilateral mobile phone use
				294	OR 1.1 (0.9-1.4)	> 1 year latency, cordless phone
				23	OR 1.8 (1.01-3.2)	> 10 years latency of cordless phone use
				11	OR **3.0** (1.3-7.2)	> 10 years latency of ipsilateral cordless phone use
Interphone Study Group (2010) 13 countries; Australia, Canada, Denmark, Finland, France, UK, Germany, Israel, Italy, Japan, New Zealand, Norway, Sweden	2000-2004, 2-4 years depending on study region. Case-control	30-59 years	Meningioma (n=2409)	1262	OR **0.79** (0.68-0.91)	Regular use of mobile phone in the past ≥1 year
				130	OR 1.15 (0.81-1.62)	Cumulative hours mobile phone ≥ 1640 hours
				21	OR 0.94 (0.31-2.86)	Cumulative hours mobile phone ≥ 1640 hours, tumors in *temporal lobe*
				46	OR 1.45 (0.80-2.61)	Cumulative hours mobile phone ≥ 1640 hours, *ipsilateral* mobile phone use

Table 2. cont.

Study	**Years Study Type**	**Age**	**Tumour type**	**No. of exposed cases**	**Odds ratio, 95 % confidence interval**	**Comments**
Interphone (2010) Appendix 2	2000-2004, 2-4 years depending on study region. Case-control	30-59 years	Meningioma (n=842)	362	OR 0.90 (0.62-1.31)	Restricted to *ever regular use* time since start 2-4 years; 1-1.9 years as reference entity
				288	OR 0.75 (0.51-1.10)	Restricted to *ever regular use* time since start 5-9 years; 1-1.9 years as reference entity
				76	OR 0.86 (0.51-1.43)	Restricted to *ever regular use* time since start 10+ years; 1-1.9 years as reference entity
				96	OR 1.10 (0.65-1.85)	Restricted to *ever regular use* ≥1640 hours, <5 hours as reference entity

Table 3. Summary of studies on the use of wireless phones and acoustic neuroma risk

Study	**Years Study Type**	**Age**	**Tumour type**	**No. of exposed cases**	**Odds ratio, 95 % confidence interval**	**Comments**
Hardell et al (2006c), Hardell, Carlberg (2009) Sweden	1997-2003 Case-control	20-80 years	Acoustic neuroma (n=243)	130	OR **1.7** (1.2-2.3)	> 1 year latency of mobile phone use
				20	OR **2.9** (1.6-5.5)	> 10 years latency of mobile phone use
				13	OR **3.0** (1.4-6.2)	> 10 years of *ipsilateral* mobile phone use
				4	OR 1.3 (0.4-3.8)	> 10 years latency of cordless phone use
				3	OR 2.3 (0.6-8.8)	> 10 years latency of *ipsilateral* cordless phone use
Sato et al (2011) Japan	2000-2006 Case-case	All ages	Acoustic neuroma (n=787)	97	RR 1.08 (0.93-1.28)	Mobile phone, reference date 1 year before diagnosis, *ipsilateral*
				86	RR 1.14 (0.96-1.40)	Mobile phone, reference date 5 years before diagnosis, *ipsilateral*
				18	RR **2.74** (1.18-7.85)	Mobile phone, reference date 1 year before diagnosis, average daily call duration >20 min, *ipsilateral*
				28	RR **3.08** (1.47-7.41)	Mobile phone, reference date 5 years before diagnosis, average daily call duration >20 min, *ipsilateral*
				45	RR 0.93 (0.79-1.14)	Cordless phone, reference date 1 year before diagnosis, *ipsilateral;* mobile phone non-users
				125	RR 1.02 (0.91-1.17)	Cordless phone, reference date 5 years before diagnosis, *ipsilateral;* mobile phone non-users

Table 3 cont.

Study	**Years Study Type**	**Age**	**Tumour type**	**No. of exposed cases**	**Odds ratio, 95 % confidence interval**	**Comments**
Interphone Study Group (2011) 13 countries; Australia, Canada, Denmark, Finland, France, UK, Germany, Israel, Italy, Japan, New Zealand, Norway, Sweden	2000-2004, 2-4 years depending on study region. Case-control	30-59 years	Acoustic neuroma (n=1105)	643	OR 0.85 (0.69-1.04)	Mobile phone regular use up to 1 year before reference date
				304	OR 0.95 (0.77-1.17)	Mobile phone regular use up to 5 years before reference date
				77	OR 1.32 (0.88-1.97)	Cumulative hours mobile phone ≥ 1640 hours up to 1 year before reference date
				36	OR **2.79** (1.51-5.16)	Cumulative hours mobile phone ≥ 1640 hours up to 5 years before reference date
				47	OR **2.33** (1.23-4.40)	Cumulative hours mobile phone ≥ 1640 hours up to 1 year before reference date; *ipsilateral* use
				27	OR **3.53** (1.59-7.82)	Cumulative hours mobile phone ≥ 1640 hours up to 5 years before reference date; *ipsilateral* use
				37	OR **1.93** (1.10-3.38)	Cumulative hours mobile phone ≥ 1640 hours in the past start ≥10 years before reference date
				28	OR **3.74** (1.58-8.83)	Cumulative hours mobile phone ≥ 1640 hours in the past start ≥10 years before reference date, *ipsilateral*
				225	OR 1.41 (0.82-2.40)	Restricted to *ever regular use* time since start 2-4 years; 1-1.9 years as reference entity
				209	OR 1.38 (0.80-2.39)	Restricted to *ever regular use* time since start 5-9 years; 1-1.9 years as reference entity
				64	OR 1.08 (0.58-2.04)	Restricted to *ever regular use* time since start 10+ years; 1-1.9 years as reference entity
				72	OR 1.74 (0.90-3.36)	Restricted to *ever regular use* ≥1640 hours, <5 hours as reference entity

Table 4. Odds ratio (OR) and 95 % confidence interval (CI) for glioma, meningioma and acoustic neuroma in different age groups for first use of the wireless phone (Hardell et al 2006b,c, 2010, 2011a). Numbers of exposed cases (Ca) and controls (Co) are given. Adjustment was made for age, gender, SEI-code, year of diagnosis. For glioma adjustment was also made for vital status.

	Glioma (n=1148)		**Meningioma (n=916)**		**Acoustic neuroma (n=243)**	
	Ca/Co	OR, CI	Ca/Co	OR, CI	Ca/Co	OR, CI
Mobile phone	529/963	**1.3** (1.1-1.6)	347/900	1.1 (0.9-1.3)	130/900	**1.7** (1.2-2.3)
< 20 years old	17/14	**3.1** (1.4-6.7)	5/14	1.9 (0.6-5.6)	5/14	**5.0** (1.5-16)
20-49 years old	315/581	**1.4** (1.1-1.7)	210/555	1.3 (0.99-1.6)	86/555	**2.0** (1.3-2.9)
≥ 50 years old	197/368	**1.3** (1.01-1.6)	132/331	1.0 (0.8-1.3)	39/331	1.4 (0.9-2.2)
Cordless phone	402/762	**1.3** (1.1-1.6)	294/701	1.1 (0.9-1.4)	96/701	**1.5** (1.04-2.0)
< 20 years old	16/16	**2.6** (1.2-5.5)	2/16	0.5 (0.1-2.2)	1/16	0.7 (0.1-5.9)
20-49 years old	206/437	1.2 (0.9-1.5)	167/416	1.3 (0.98-1.6)	65/416	**1.7** (1.1-2.5)
≥ 50 years old	180/309	**1.4** (1.1-1.7)	125/269	1.1 (0.8-1.4)	30/269	1.3 (0.8-2.1)

Table 5. Gender and age distribution for use of mobile phones among cases aged 20-80 years in the Hardell group studies. Glioma (n=1148).

	Men		**Women**		**Total**	
Age, diagnosis	No use/≤1 year latency, mobile phones	Use >1 year latency, mobile phones	No use/≤1 year latency, mobile phones	Use >1 year latency, mobile phones	No use/≤1 year latency, mobile phones	Use >1 year latency, mobile phones
20-24	8	7 (47 %)	3	8 (73 %)	11	15 (58 %)
25-29	10	15 (60 %)	5	10 (67 %)	15	25 (63 %)
30-34	11	26 (70 %)	19	8 (30 %)	30	34 (53 %)
35-39	9	23 (72 %)	8	13 (62 %)	17	36 (68 %)
40-44	10	26 (72 %)	16	11 (41 %)	26	37 (59 %)
45-49	14	37 (73 %)	12	16 (57 %)	26	53 (67 %)
50-54	22	61 (73 %)	26	27 (51 %)	48	88 (65 %)
55-59	35	65 (65 %)	59	20 (25 %)	94	85 (47 %)
60-64	41	51 (55 %)	53	15 (22 %)	94	66 (41 %)
65-69	55	46 (46 %)	57	13 (19 %)	112	59 (35 %)
70-74	43	16 (27 %)	41	5 (11 %)	84	21 (20 %)
75-80	27	8 (23 %)	35	2 (5 %)	62	10 (14 %)
All	**285**	**381 (57 %)**	**334**	**148 (31 %)**	**619**	**529 (46 %)**

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 11

Evidence for Brain Tumors (Epidemiological)

Supplement 2012

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I. INTRODUCTION

Primary central nervous system (CNS) tumors are a heterogeneous group of benign and malignant neoplasms localized in the brain, the spinal cord and their coverings. They differ in histological type, tissue of origin, anatomic site, growth pattern, age distribution, sex ratio, clinical appearance and many other features including molecular neuropathological markers. These features are not independent but little is known about the etiology of these tumors and the reason for the observed epidemiological patterns. The rapidly developing field of molecular neuropathology may provide clues to solve these problems in the future.

Annually about 57,000 new cases of CNS tumors are diagnosed in the US. The age distribution has two peaks: incidence is about 4.7 cases per 100,000 per year below 10 years of age (which is mainly due to astrocytoma of the juvenile pilocytic type, malignant glioma, medulloblastoma and tumors originating from mesodermal and embryonic tissues), and after age 15 there is a steady increase of incidence with increasing age reaching its second peak of about 68 cases per 100,000 per year at an age around 75 to 80 years (CBTRUS, 2011). The burden of CNS cancers is distinctly higher in children making up around 20% of all childhood malignancies, while in adults less than 2% of all cancers are primary brain cancers.

There are some rare cases of inherited cancer syndromes (e.g. von Hippel-Lindau disease, Li-Fraumeni syndrome) that are related to brain tumor risk, accounting for a small fraction of cases. Except for therapeutic x-rays no environmental or lifestyle factor has unequivocally been established as risk factor for brain tumors. Non-whites seem to have lower risk, and incidence tends to be higher with increasing socio-economic status. However, because of the rather advanced age of 75-80 years of peak incidence, such differences may partly be due to differences in life-expectancy. During the last decades of the 20th century some types of brain tumors show a steady increase of a few percent per year, which might to some extent be related to the introduction of computed tomography and other high-resolution neuroimaging methods. For most CNS tumors except meningioma and pituitary tumors the incidence is higher in males than females.

Since the report of Wertheimer and Leeper in 1979 of an increased incidence of brain tumors in children living in homes with an expected higher exposure to power-frequency electric and magnetic fields, exposure to electromagnetic fields have become an area of interest in the study of factors affecting brain tumor risk.

This review focuses on the radio frequency (RF) part of the electromagnetic spectrum (3 kHz to 300 GHz). However, because the epidemiology of mobile phone use is covered in another section, it will be restricted to RF exposure conditions other than microwaves from mobile phone use. Exposure to ELF magnetic fields and childhood brain tumors is covered in the chapter about childhood cancers.

II. MATERIAL AND METHODS

Published articles of relevant studies restricted to the years 1987 to 2012 were obtained by searching PubMed using the following terms:

(“radio frequency” OR electromagnetic* OR microwaves) AND (“brain cancer” OR brain tumor* OR “CNS cancer” OR CNS tumor* OR glioma* OR meningioma* OR neuroma*) NOT (“power frequency” OR “low frequency”) AND epidemiolog*

The search resulted in 137 hits. After removing reviews and animal or in vitro studies as well as studies of mobile phone use, 10 articles remained. A hand search in review papers (Krewski et al. 2001; Elwood 2003; Ahlbom et al. 2004; Kundi et al. 2004) and reference lists of the articles found in PubMed revealed another 9 papers; hence the final body of evidence consists of 19 studies of exposure to various types of RF fields.

Of the 19 studies 8 were cohort studies, 5 case-control studies and 6 of an ecological type. The majority of studies (11) were occupational studies, four studies investigated children, and one ecological study investigated both, adults and children.

III. EPIDEMIOLOGICAL STUDIES OF RF FIELDS AND BRAIN TUMORS

Table 10A-1 gives an overview of the 17 studies obtained by the literature search with respect to study type, assessment of exposure and outcome, confounders considered and matching variables used, number of cases included and selection method of study participants. Results are summarized in Table 10A-2.

Table 10A- 1: Synopsis of epidemiologic studies of or including brain tumors (1987 – 2007)

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Thomas et al. 1987	Northern New Jersey, Philadelphia, gulf coast of Lousiana/1979-1981/Case-control	Interviews with next-of-kin about occupational history – response rates: cases 74%, controls 63%; JEM (2 methods)	Death certificates verified through review of hospital records	age(m), (only males), year of death(m), area of residence(m), educational level, (lead, soldering fumes)	435/386	Cases: deaths of brain tumor or CNS tumors of white males (age>30) from death certificates Controls: deaths from other causes than brain tumors, epilepsy, etc.
Milham 1988	Washington, California/1979-1984/Cohort	Amateur radio operator license within 1/1979 to 6/1984	Mortality records	age, (only males), race, year of death	29	67829 operators, search of deaths in state registry through 1984
Selvin et al. 1992	San Francisco/1973-1988/Spatial cluster	Distance of center of census tract to microwave tower (Sutro tower)	SEER records	-	35	Search of cancer deaths of white individuals (age<21)

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Tynes et al. 1992	Norway/1961-1985 /Occupational cohort	Job title in 1960 and 1970 censuses and expert categorization	Cancer registry	age, (only males)	119 overall, 6 in subgroup with possible RF exposure	Cohort of 37945 male workers identified that had jobs in 1960 with possible EMF exposure. among these 3017 with possible RF exposure
Grayson 1996	US Air Force/1970-1989/Nested case-control	Detailed job history and classification based on JEM (RF/MW exposure from frequent measurements)	Screening of hospital discharge records	age(m), race(m), military rank, (ELF and ionizing radiation exposure)	230/920	Cohort of ~880000 US Air Force members with at least one completed year of service within the study period, no follow up after subjects left service
Szmigielski 1996	Poland (military)/1971 -1985/Occupational cohort	Allocation to RF/MW exposure group based on service records, documented measurements of military safety groups	Incident cases from central and regional military hospitals and military health departments	age, (only males)	~46	Annual number of ~127800 military career personnel, ~3720 RF/MW exposed per year

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Hocking et al. 1996	Sydney (Australia)/ 1972-1990/Ecological	Municipalities within ~4 km of 3 TV broadcasting towers considered higher exposed as compared to 6 further away	Incident and death cases from cancer registry	age, sex, calendar period	740 (incident) 606 (mortality) 64 age<15 (incident) 30 age<15 (mortality)	Study population: inner area ~135000, outer area ~450000
Tynes et al. 1996	Norway/1961-1991/ Occupational cohort	Certified radio and telegraph operators 1920-1980 (98% worked on merchant ships); spot measurements on ships with old-fashioned equipment	Cancer registry	age, (only females)	5	2619 women certified as radio or telegraph operators by Norwegian Telecom
Dolk et al. 1997a	Birmingham (GB)/ 1974-1986/Ecological	Living near a TV/FM radio transmitter (Sutton Coldfield)	Cancer registry	age, sex, calendar year, SES	332	Population (age≥15) ~408000 within 10 km of the transmitter
Dolk et al. 1997b	GB/1974-1986/	Living near a	Cancer registry	age, sex,	244	Population (age<15)

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
	Ecological	high power (≥500 kW erp) transmitter (overall 21)		calendar year, SES		within 10 km of one of 20 high power transmitters
Lagorio et al. 1997	Italy/1962-1992/ Occupational cohort	Working as RF heat-sealer operator	Cancer deaths from registry	age, (only females), calendar period, region	1	302 women employed 1962-1992 in a plastic-ware manufacturing plant as RF sealers
Finkelstein 1998	Ontario (Canada)/ 1964-1995/ Occupational cohort	Working as a police officer (possible handheld radar exposure)	Cancer registry	age, (only males), calendar year	16	20601 male officers of Ontario Police
Morgan et al. 2000	USA/1976-1996/ Occupational cohort	Jobs classified according to work with RF emitting devices with different output power	Death certificates from states' statistics offices	age, sex, period of hire	51	All U.S. Motorola employees with at least 1 day employment 1976-1996 (195775 workers, 2,7 million person-years)

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Groves et al. 2002	USA/1950-1997/ Occupational cohort	6 occupational groups 3 with assumed low radar exposure (radar-, radio operator, aviation electrician's mate) and 3 with assumed high exposure (aviation electronics -, electronics -, fire control technician)	Death certificate from a state vital statistics office or National Death Index Plus	age at entry, (only males), attained age	88	40581 Navy Korean War veterans graduated 1950-54 from Navy technical schools; follow-up from graduation through 1997
Ha et al. 2003	South Korea/1993-1996/Ecological	Area <2 km around 11 high power and 31 low power AM radio transmitter and control areas >2 km from any transmitter	Cancer cases from insurance records	age, sex (direct and indirect standardization)	45/not specified	Census and residents registration data 1995 (population size between 3152 and 126523 at the different sites)
Park et al. 2004	South Korea/1994-1995/Ecological	10 areas with a AM radio transmitter ≥100kW	Cancer deaths from death certificates	age, sex (direct standardization)	30/100	Census data from 1990

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
Berg et al. 2006	Germany/2000-2003/ Case-control	JEM from occupational history collected in interview	Histological verified cases of glioma and meningioma	age(m), sex(m), region(m), SES, urban/rural, smoking, ionizing rad. exposure	Glioma 366/732 Meningioma 381/762	All histological confirmed cases of glioma and meningioma from 4 neurosurgical clinics (age: 30-69) (part.rate 84%); frequency matched controls from population registry (part.rate 63%)
Schüz et al. 2006	Germany/2000-2003/ Case-control	Questionnaire about DECT cordless phone base station near the bed	Histological verified cases of glioma and meningioma	age(m), sex(m), region(m), SES, urban/rural, smoking, ionizing rad. exposure	Glioma 366/732 Meningioma 381/762	All histological confirmed cases of glioma and meningioma from 4 neurosurgical clinics (age: 30-69) (part.rate 84%); frequency matched controls from population registry (part.rate 63%)
Ha et al. 2007	South Korea/1993-1999/Case-control	Distance from 31 AM radio transmitters and 49 radio antennas, measurements and calculation of	Cases of brain cancer from verified by entry into cancer registry	age(m), sex(m), year of diagnosis(m), SES, population density	956/1020	All cases of brain cancer (age<15) from 14 hospitals and matched hospital controls with respiratory diseases

Study	Country/Period/Study Type	Exposure assessment	Outcome assessment	Confounders considered & matching variables(m)	Number of cases/controls or cases (cohort studies)	Selection of participants
		total RF electric field strength				

SES…socio-economic status, JEM…job exposure matrix, erp…equivalent radiation power, RF/MW…radio frequency/microwaves, CNS…central nervous system, ELF…extremely low frequency

Table 10A- 2: Synopsis of main results of brain tumor studies (1987 – 2007)

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
Thomas et al. 1987	Brain tumor deaths (ICD not specified)	Ever exposed to RF	OR	1.6 [1.0 – 2.4]
		Electrical/electronics job	OR	2.3 [1.3 – 4.2]
		Unexposed*		
		Ever exposed < 5 y	OR	1.0
		5-19 y	OR	2.3
		20+ y	OR	2.0
Milham 1988	Brain cancer deaths (ICD-8: 191)	All	SMR	1.39 [0.93 – 2.00]
		Novice[a]	SMR	0.34
		Technician	SMR	1.12
		General	SMR	1.75
		Advanced	SMR	1.74
		Extra	SMR	1.14
Selvin et al. 1992	Brain cancer deaths (ICD-O: 191.2)	> 3.5 km distance from tower*		
		≤ 3.5 km[b]	RR	1.16 [0.60 – 2.26]
Tynes et al. 1992	Incident brain cancer (ICD-7: 193)	All with possible EMF exposure	SIR	1.09 [0.90 – 1.41]
		Subgroup possible RF exposure[c]	SIR	0.49 [0.18 – 1.06]
Grayson 1996	Incident brain cancer (ICD-9: 191)	Never RF/MW exposed*		
		Ever exposed	OR	1.39 [1.01 – 1.90]
Szmigielski 1996	Incident nervous system & brain tumors	RF/MW exposed	OER	1.91 [1.08 – 3.47]
Hocking et al. 1996	Brain cancer (ICD-9: 191)	Outer area*		
		Inner area (incident, overall)	RR	0.89 [0.71 – 1.11]
		Inner area (mortality, overall)	RR	0.82 [0.63 – 1.07]
		Inner area (incident, age<15)	RR	1.10 [0.59 – 2.06]
		Inner area (mortality, age<15)	RR	0.73 [0.26 – 2.10]
Tynes et al. 1996	Incident brain cancer (ICD-7: 193)	All	SIR	1.0 [0.3 – 2.3]
Dolk et al. 1997a	Incident brain tumors (ICD-8/9: 191, 192)	0-2 km from transmitter	OER	1.29 [0.80 – 2.06]
		0-10 km from transmitter	OER	1.04 [0.94 – 1.16]
Dolk et al. 1997b	Incident brain tumors (ICD-8/9: 191, 192)	0-2 km from transmitter	OER	0.62 [0.17 – 1.59]
		0-10 km from transmitter	OER	1.06 [0.93 – 1.20]

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
Lagorio et al. 1997	Brain cancer deaths (ICD-9: 191)	RF sealer operator	OER	1 : 0.1
Finkelstein 1998	Incident brain cancer (ICD-9: 191)	All police officers	SIR	0.84 [0.48 – 1.36]
Morgan et al. 2000	Incident brain cancer (ICD-9: 191)	No RF exposure*		
		Low[d]	RR	0.92 [0.43 – 1.77]
		Moderate	RR	1.18 [0.36 – 2.92]
		High	RR	1.07 [0.32 – 2.66]
Groves et al. 2002	Brain cancer deaths (ICD-9: 191)	Low radar exposure*		
		High radar exposure	RR	0.65 [0.43 – 1.01]
Ha et al. 2003	Brain cancer (ICD-10:C70-C72)	Low power transmitters*		
		High power transmitters	SIR	1.8 [0.8 – 11.1]
		Control sites (>2 km)*		
		100 kW transmitter	OER	2.27 [1.30 – 3.67]
		250 kW	OER	0.86 [0.41 – 1.59]
		500 kW	OER	1.47 [0.84 – 2.38]
		1500 kW	OER	2.19 [0.45 – 6.39]
Park et al. 2004	Brain cancer deaths (ICD-10:C69-C72)	Control area*		
		≥100 kW transmitter	SMR	1.52 [0.61 – 3.75]
Berg et al. 2006	Incident glioma (ICD-O3: C71)	No occup. RF/MW exposure*		
		Probably no exposure	OR	0.84 [0.48 – 1.46]
		Probable exposure	OR	0.84 [0.46 – 1.56]
		High exposure	OR	1.22 [0.69 – 2.15]
		No high exposure*		
		High exposure <10 y	OR	1.11 [0.48 – 2.56]
		High exposure ≥ 10 y	OR	1.39 [0.67 – 2.88]
	Incident meningioma (ICD-O3: C70.0)	No occup. RF/MW exposure*		
		Probably no exposure	OR	1.11 [0.57 – 2.15]
		Probable exposure	OR	1.01 [0.52 – 1.93]
		High exposure	OR	1.34 [0.61 – 2.96]
		No high exposure*		
		High exposure <10 y	OR	1.15 [0.37 – 3.48]

Study	Endpoint	Exposure category	Meas.	Outcome [95% CI]
		High exposure ≥ 10 y	OR	1.55 [0.52 – 4.62]
Schüz et al. 2006	Incident glioma (ICD-O3: C71)	DECT near bed	OR	0.82 [0.29 – 2.33]
	Incident meningioma (ICD-O3: C70.0)	DECT near bed	OR	0.83 [0.29 – 2.36]
Ha et al. 2007	All brain cancers (ICD-10: C70-C72)	≤2 km	OR	1.42 [0.38 – 5.28]
		2-4 km	OR	1.40 [0.77 – 2.56]
		4-6 km	OR	1.02 [0.66 – 1.57]
		6-8 km	OR	1.08 [0.73 – 1.59]
		8-10 km	OR	0.94 [0.67 – 1.33]
		10-20 km	OR	1.01 [0.77 – 1.34]
		>20 km*		

[*] Reference

[a] From Milham 1988b, license classes as proxy for exposure duration

[b] Based on the assumption that exposure is higher near the microwave tower

[c] Computed based on Table 5 in Tynes et al. 1992

[d] Classification according to power output of equipment used for longest period of employment

OR…odds-ratio, SIR…standardized incidence ratio, SMR…standardized mortality ratio, RR…relative risk (rate ratio), OER…observed/expected ratio

In the following paragraphs each study is briefly discussed with respect to its strengths and weaknesses.

A. Thomas et al. 1987

This case-control study included 435 deaths from brain or CNS tumors and 386 deaths from other causes as controls. Only adult males were included. Basis of data collection on occupational history were interviews with next-of-kin. Two methods of classification were used: one method assigned subjects to one of three categories (never exposed to RF/ever exposed to RF in an electrical or electronics job/ever exposed to RF but not in an electrical or electronics job), the other method consisted of a classification of each job by an industrial hygienist for presumed exposure to RF, soldering fumes, and lead. Both methods revealed significantly increased brain tumor risks of presumed occupational exposure to RF fields. This increase was due to an association in electronics and electrical jobs with astrocytic tumors as the predominant outcome associated with employment in these categories. In addition a significant increase of brain tumor risk was found for increasing duration of exposure.

Although relying on information of next-of-kin could be a source of misclassification, one strength of this study is it's relying on occupational history only that could be assumed to be more accurate than recall of exposure to various agents. The two methods of classification led to almost the same results, which lends support to the hypothesis that indeed exposure in electrical and electronics jobs is associated with an increased brain tumor risk. Due to the relationship between RF exposure and exposure to lead, solvents or soldering fumes in these jobs, it is not possible to separate effects of these exposures. Soldering fumes were never investigates with respect to brain tumors, and the hypothesis of an association with sinonasal cancer could not be corroborated so far. However, analysis of exposure to lead did not show a consistent relationship with brain tumor risk, indicating that it may not confound the relationship to RF exposure.

Because this study is of dead cases only it is likely over-representing high grade brain tumors that may not all be associated with exposure leading to an effect dilution. Exposure misclassification, if it is non-differential in cases and controls, also reduces effect estimates.

A weakness of this study is obviously its lack of an exposure indicator other than the occupational category. While there is no doubt that in these jobs some exposure to RF fields occur quite regularly, specific characteristics including frequency ranges, modulation, intensity, duration and distance from the source vary considerably. Overall the study (as well as two earlier ones outside the search window: Lin et al. 1985 and Milham 1985) are sufficient to formulate a research hypothesis that can be tested in appropriately designed subsequent investigations. Unfortunately such studies have never been conducted.

B. Milham 1988

In this cohort study of 67,829 amateur radio operators holding a license within 1/1979 to 6/1984 in Washington and California 29 brain tumor deaths occurred during the follow up period with 21 expected.

It should be noted that there was a substantial and statistically significant lower number of overall deaths of less than three quarters of deaths expected from country mortality rates. This could be due to both a ‘healthy-worker’ effect as well as an effect of socio-economic status. In lieu of computing standardized mortality ratios (SMR) it may be instructive to look at the proportional mortality rates in the reference population and the amateur radio operators: 0.6% of all deaths are expected to be due to brain tumors in the reference population while in amateur radio operators twice as many occurred (1.2%). Whether or not this is an indication of an increased brain tumor risk due to RF exposure is difficult to assess. First of all, this study is a register only investigation and no information on intensity, frequency and duration of engagement in amateur radio operations were available. In a later analysis the author reported about results using a proxy of intensity and duration of exposure: the license class. In this analysis indications of an increase of risk with increasing license class were obtained.

This study could and should have started off a thorough follow up of amateur radio operators and nested case-control studies to address the problem of potential confounders and to narrow down the conditions that may be responsible for the increased mortality from some cancers. It is another loose end that leaves us without a clear message.

Although no risk factor for brain cancer except therapeutic ionizing radiation is known, there are some indications that risk increases with social class. The reason for this association is unknown but life-style factors may play a role as well as concomitant causes of death that could lead to a spurious reduction of risk in lower class populations because brain tumors have their peak close to life-expectancy.

C. Selvin et al. 1992

The objective of this investigation was not primarily to study the relationship between RF exposure and childhood cancer but to address the general problem of how to assess disease incidence or mortality in relation to a point source. As the point source the Sutro Tower in San Francisco, the only microwaves emitting tower in this county, was chosen. A total of 35 brain tumor deaths occurred among 50,686 white individuals at risk aged less than 21 in the years 1973-88 in an area of approximately 6 km around the tower. The exact location of residence could not be obtained; therefore each case was located in the center of the census tract. Different methods of analysis were applied to assess a potential relationship between distance from the tower and brain tumor risk. Relative risk for brain tumors for a distance less than 3.5 km from Sutro Tower compared to more than 3.5 km was 1.162 and not significant.

The study explored different methodological procedures and has its merits from a methodological point of view. However, it starts from the wrong assumption: that distance to a point source is a valid proxy for intensity of exposure. Under ideal conditions of spherical symmetry of an emission this assumption holds, however, there are almost no real life situations where this assumption is sufficiently close to actual exposure levels. And it is definitely not true for the Sutro Tower. Radiations from the antennae are directed towards the horizon and the complex pattern of emission with main and side lobes results in a complex pattern of RF exposure at ground level. Furthermore, the area is topographically structured with hills and valleys such that areas of high exposure at the vertices are in close proximity to areas of low exposure at the shadowed side downhill.

Studying the relationship between a point source and disease is not only difficult due to the complex relationship between distance and exposure but also because of the fact that humans are not stable at a certain location. This is of greater importance for adults who may commute from and to work places and have generally a greater radius of activity as compared to children. Nevertheless, there is at least a high chance of one long-lasting stable location that is when people sleep in their beds. Therefore, studies in relation to a point source should attempt to assess exposure at the location of the bed. Because the objective of this study was not the assessment of a potential brain tumor risk but the application of methods for the analysis of spatial data, no attempts were made to measure actual exposure.

D. Tynes et al. 1992

In this study information on occupations obtained for all Norwegians every 10 years was used to assess cancer incidence in relation to job titles. In 1960 37,945 male workers were identified that had jobs with possible exposure to EMFs and among these 3,017 with possible RF exposure. Overall 119 brain tumor cases were found in the cancer registry between 1961 and 1985. Of these cases 6 occurred in the subgroup of workers possibly exposed to RF fields. The overall expected number of brain tumor cases was 109 and 12 for the subgroup with possible RF exposure. Hence no increased brain tumor risk could be detected.

Despite the long follow-up period of 25 years with an accumulated number of 65,500 person-years the expected number of brain tumors diagnosed during that period is too low to detect a moderately elevated risk of 1.3 to 1.5. Furthermore, the follow up period just reaches the median induction period for brain tumors as delineated from studies on ionizing radiation.

As mentioned above, all studies solely relying on job titles lead to exposure misclassification and, therefore, to a dilution of risk. For dichotomous exposure variables (exposed/not exposed) and assuming a negligibly small proportion of exposed in the reference population standardized incidence ratios (SIR) are biased by a factor (1+f*(SIR-1))/SIR, if f denotes the fraction of true exposed and SIR is the true incidence ratio. Hence a true SIR of 2.0 is reduced to 1.5 if only 50% in the cohort are actually exposed. The observed SIR is further reduced if the assumption of a negligible fraction of exposed in the reference population is wrong. In this case the bias factor given above is further divided by (1+g*(SIR-1)), where g is the fraction of exposed in the general population.

While a cohort study that is based on registry data has the advantage of independence from recall errors and selection bias due to possible differential participation, it has the disadvantage that registry data are generally insufficient to provide reliable exposure indicators. While no association with brain tumors could be detected in this study it revealed an increased number of leukemia cases in occupations with possible RF exposure. This could be due to the higher incidence of leukemia or to a stronger association or to the shorter latency and various other reasons including chance.

E. Grayson 1996

In this case-control study nested within approx. 880,000 US Air Force personnel with at least one years of service during the study period of 1970-89, primary malignant brain tumor cases were ascertained by screening hospital discharge records. The study included only males and only as long as they were on Air Force records. From 246 cases detected 16 were dropped due to incomplete or ambiguous data. For each case four controls were randomly selected from the case's risk set matching it exactly on year of birth and race. Controls that were diagnosed with diseases possibly associated with EMF exposure (leukemia, breast cancer, malignant melanoma) were excluded from the risk set.

A strength of this study is the detailed job history filed for each cohort member that could be used for retrospective exposure assessment. Furthermore, Air Force files contained detailed data from personal dosímetry on ionizing radiation for the different posts and jobs. Classification of RF field exposure was based on a detailed job exposure matrix with over 1,950 entries, indexing 552 different job titles. One source of classification was recorded events of exposure to RF fields above 100 W/m^2. By this method probable exposure was assigned if for a job such events were recorded in the past as well as for closely related jobs. Possible exposure was assigned for jobs that required operation of RF emitters but without recorded overexposure.

A further strength is the thorough consideration of possible confounders. Because of the possible relationship of brain tumor risk with socio-economic status (SES), military rank was used as a surrogate for SES and included in the analysis as well as ionizing radiation exposure that has previously been shown to increase brain tumor risk.

Exposure to RF fields was associated with a moderate but statistically significant increased risk of OR=1.39. Investigation of duration of exposure was compromised by an ambiguity introduced due to the calculation of an exposure score as the product of exposure and months. Nevertheless, for those ever exposed there were indications of an increasing risk with increasing exposure duration.

A weakness of this investigation is its incomplete follow-up of cohort members. This could have resulted in an underestimation of the true risk. Leaving the Air Force could have been more likely in those exposed to RF fields and developing a brain tumor. Some malignant brain tumors have early signs that could be incompatible with the Air Force job especially if involving operation of RF equipment (like seizures, severe headaches, somnolence, and absences). Because the study did not involve personal contact it is free of other selection biases.

F. Szmigielski 1996

In this military cohort study of cancer morbidity Polish military career personnel was assessed for occupational exposure to RF fields based on service records. The study covered 15 years (1971-85) including approx. 128,000 persons per year. Expected rates for 12 cancer types were calculated based on the age specific morbidity in those classified as unexposed.

For brain and nervous system tumors a significantly increased ratio of observed to expected (OER=1.91) was found. Other malignancies with significantly increased incidence in exposed were: esophageal and stomach cancers, colorectal cancers, melanoma, and leukemia/lymphoma.

A strength of this study is its substantial size with almost 2 million person-years of follow-up. Furthermore, accurate military records on job assignment and on exposure from military safety groups gives a unique opportunity to assess long-term exposure effects based on already filed data.

Some important data are missing because they were military classified information that could not be provided in the paper. This includes the exact number of cases of the different neoplasms. However, from the data presented an observed number of brain tumors of about 46 can be calculated.

The study has been criticized for an alleged bias because more information on risk factors was available for cancer cases. It is true that military medical boards collected data for cases such as life style factors and exposure to possible carcinogens during service, however, at no stage this information entered the analysis. Therefore, this criticism is unfounded. Such information could have been utilized within a nested case-control study applying the same methods of assessment of risk factors for controls as has been done for cases. Because some findings, such as the increased risk for esophagus/stomach cancer, that are rarely reported in relation to RF exposure warrant further study, such a nested case-control approach is recommended. It could, albeit with some difficulties, even be successfully conducted retrospectively.

G. Hocking et al. 1996

In an ecological study cancer incidence and mortality in nine municipalities of northern Sydney during 1972-90 three of which surround three TV towers were assessed. Population size in the three municipalities located within a radius of approx. 4 km around the TV towers amounts to 135,000, while population size in the six municipalities further away was 450,000. High-power transmission commenced in 1956, an additional 100 kW transmission started in 1965 and another 300 kV broadcast in 1980. Carrier frequencies varied between 63 and 533 MHz for TV broadcasting and were around 100 MHz for FM radio broadcast.

During the study period 740 primary malignant brain tumors were diagnosed in adults and 64 in children, 606 deaths due to brain cancer occurred in adults and 30 in children. While incidence of lymphatic leukemia was significantly higher in adults as well as in children inhabiting the three municipalities surrounding the transmission towers compared to the six districts further away, brain tumor incidence was not significantly elevated (RR=0.89 in adults and 1.10 in children).

As has been stated above, distance from a transmitter is a poor proxy for exposure. Some measurements done in the study area obtained levels much lower than those calculated from the power emitted and antenna gain. Several factors are responsible for this effect: multiple reflections, attenuation by buildings and vegetation, ground undulations, non-coincidence of maxima for the different signals as well as complex radiation characteristics of the broadcast antennae.

The exact location of the residence of cases could not be provided which reduces the potential of the study to relate incidences to measurements or calculations of RF fields. Authors discussed some potential sources of bias such as migration and other exposures in the different regions. However, the most important disadvantage in such studies is that individual risk factors cannot be adjusted for. Both spurious positive as well as false negative results can be obtained by disregarding such individual variables.

H. Tynes et al. 1996

In a historical cohort study 2,619 Norwegian female radio and telegraph operators certified between 1920 and 1980 were followed from 1961 through 1991 for entries in the cancer registry. During this period a total of 140 cases of cancer occurred which are about 20% more than expected from the Norwegian population. Among these were 5 brain tumor cases closely matching the number expected.

An excess for breast cancer was found in this study that may be related to a combination of RF field exposure and night work. For other cancers including brain cancer numbers of cases were too low to address exposure risk.

In this very thoroughly conducted study including a nested case-control approach for breast cancer, measurements at historical transmitters on ships, comparison with women at other jobs on sea, brain tumors were not distinctly higher than expected from the reference population. However, because of the limited cohort size a moderately increased risk cannot be excluded.

I. Dolk et al. 1997a

This ecological small area study of cancer incidence 1974-86 near the Sutton Coldfield TV/radio transmitter at the northern edge of the city of Birmingham (England) was initiated by an unconfirmed report of a ‘cluster’ of leukemias and lymphomas. The transmitter came into service in 1949. Transmission at 1 megawatt (effective radiated power erp) began in 1964, at 3 MW in 1969, and at 4 MW in 1982. The tower has a height of 240 m with no big hills in the surrounding area. The study area was defined by a circle of 10 km radius centered at the transmitter. The population within this area was about 408,000. All cancers, excluding non-melanoma skin cancer, were considered focusing on hematopoietic and lymphatic cancers, brain and nervous system cancers, eye cancer, and male breast cancer. Childhood cancers were restricted to all cancers and all leukemias.

In the study area a small but significant excess of all cancers was observed in adults. All leukemias and non-Hodgkin’s lymphoma were particularly elevated and incidence within 2 to 4 km from the tower was about 30% higher than expected. Brain tumors were only analyzed for distances of within 2 km and the whole study area. Within 2 km an increased OER of 1.29

for all brain tumors and 1.31 for malignant brain tumors was calculated based on 17 and 12 cases, respectively.

Also this investigation suffers from using distance from the tower as proxy for intensity of exposure. The wrong assumption that exposure decreases with increasing distance invalidates the statistical trend test applied. Measurements conducted in the study area revealed the poor relationship with distance but without consequences on the evaluation of the data. Overall the study is consistent with a moderately increased risk of hematopoietic and lymphatic cancers as well as some other cancers including brain cancer in the vicinity of high-power transmitters that, if related to RF fields, must be substantially higher for actual exposure.

The Sutton Coldfield study was later continued (Cooper & Saunders 2001) to cover the period 1987-94. The study revealed, compared to the earlier period, an almost unchanged increase of leukemias and non-Hodgkin’s lymphoma in adults and a slight increase in children.

J. Dolk et al. 1997b

Because the Sutton Coldfield study was triggered by a cluster report and to provide independent test of hypotheses arising from that study, similar methods as applied in the previous study were used to study all high-power TV/radio transmitters (≥ 500 kW ERP) in Great Britain. In adults leukemias, bladder cancer, and skin melanoma, and in children, leukemias and brain tumors were studied. The study period was 1974-86 for England and somewhat shorter in Wales and Scotland.

Although population density around transmitters was not always as high as in the case of the Sutton Coldfield tower, with an average population density of only about one third of that around Sutton Coldfield tower within 2 km from the towers, in the most important range of 2 to 4 km from the transmitters, where in many cases the maximum of radiated RF at ground level is reached, population density was similar. The study of all high-power transmitters essentially corroborated the findings for adult leukemias with an increase of incidence between 10 and 50% in the distance band of 2 to 4 km from the transmitters for the different transmitter types. Most of these increased incidences were statistically significant.

For children only the incidence in the whole study area and within a distance of 2 km was calculated, which is unfortunate because the area close to the towers is sparsely populated and exposure is low. Number of brain tumors in children was slightly above expectation (244 observed and 231 expected).

In contrast to the interpretation by the authors, the study of all high power transmitters essentially replicated and supported the findings of an excess incidence of leukemias in relation to RF emission from TV/radio towers. Because the different heights and radiation characteristics of the transmitters result in different exposure patterns at ground level, the consistent increase in an area that is likely close to the maximum of exposure supports the hypothesis of an association.

K. Lagorio et al. 1997

A mortality study of a cohort of 481 female plastic-ware workers employed between 1962 and 1992 in an Italian plant, 302 of which were engaged in the sealing department with exposure to RF fields, was reported by Lagorio et al. (1997). For RF-sealers 6,772 person-years of follow-up were accumulated and overall 9 deaths occurred, 6 of which were from malignant neoplasms (which are twice as many as expected from comparison with the local reference population). In the 31 years only one brain cancer occurred but only 0.1 were expected.

Although the small size of the cohort and the potential exposure to other agents except RF fields such as solvents and vinyl chloride prohibit far reaching conclusion, much more of such thorough follow-up studies of exposed cohorts are needed to accumulate a body of evidence that can provide a useful basis for analysis.

L. Finkelstein 1998

A preliminary study intended to form the basis for an assessment of cancer risks associated with handheld radar devices was conducted among a cohort of 20,601 male Ontario police officers. The retrospective follow up covered the period of 1964-95. By linkage with the cancer registry and mortality database 650 cases of cancer were detected.

Testicular cancer and melanoma showed an excess incidence while overall cancer incidence was reduced as expected from a working cohort. Overall 16 cases of primary malignant brain tumors occurred which is slightly less than expected.

The author had difficulties to build up a proper cohort because some departments refused to participate and others couldn't spare the time to provide lists of all officers employed during the target period. Furthermore, while cancer sites of primary interest showed actually an increased incidence calling for a nested case-control approach, this study was never conducted due to lack of interest and support of the authorities.

M. Morgan et al. 2000

In an occupational cohort study all US Motorola employees with at least 6 months cumulative employment and at least 1 day of employment in the period 1976-96 were included. A total of 195,775 workers contributing about 2.7 million person-years were available for the study. The cohort was compared to the SSA Master Mortality File and the National Death Index to obtain vital status. Death certificates were obtained by states' vital statistics offices and company records. Exposure was assessed by expert opinion. Four RF exposure groups were defined with increasing level of estimated RF exposure. Only about 5% of the total cohort was classified as highly exposed and more than 70% with only background exposure. Neither private nor occupational mobile phone use was included.

Overall 6,296 deaths occurred in the cohort in 21 years, which were only two thirds of deaths expected from mortality data of the four countries where most Motorola facilities are located. This reduction is too pronounced to be solely due to a healthy worker effect, other factors such as higher SES must have contributed, an interpretation supported by the substantial reduction of mortality from all life-style associated causes of death. Internal comparisons were done for mortality from brain cancer and hematopoietic and lymphatic cancers. Brain tumor mortality was slightly but insignificantly elevated in high and moderately high exposed workers as compared to those with no or low RF exposure.

This study of a huge cohort demonstrates the limitations of such a study design. The majority of the cohort (58%) consisted of retired or terminated workers that may or may not have accumulated further RF exposure at other companies. Furthermore, it can be assumed that Motorola employees were among the first that used mobile phones at the workplace and privately. Neglecting mobile phone use may diminish the gradient of exposures between occupational groups studied. It would have been better to conduct nested case-control studies instead of using internal comparison that may be compromised by mobility bias, exposure misclassification and use of mobile phones.

N. Groves et al. 2002

In this military cohort study of 40,581 men followed from the year of graduation (1950-1954) from Navy technical schools through 1997, known as the Korean War Veterans study, groups of sailors with imputed difference in likelihood and amount of exposure to radar waves were compared with respect to mortality. The original study, with a follow up through 1974, (Robinette et al. 1980) reported increased risks of cancer of the hematopoietic and lymphatic system, of the lung and digestive system for the high exposure group but was handicapped by the lack of information on date of birth of the cohort members. For the extended follow up study many missing birth dates were found in the Veterans Administration Master Index. Nevertheless, birth date remained unknown for over 8% of the cohort. Based on expert opinion low RF exposure was assigned to job classifications of radioman, radarman, and aviation electrician's mate, high exposure stratum included men with job classifications of electronics technician, aviation electronics technician, and fire control technician.

By matching against the Social Security Administration's Death Master File and the National Death Index 8,393 deceased subjects were identified through 1997. This number is substantially and significantly lower as expected from the male white US population. A healthy soldier effect may have been responsible for a lower mortality rate in the 1950ies but cannot explain the reduced mortality after 40 years. It has not been reported how long the cohort members stayed in service nor were life-style factors investigated; however, of more than 40% of the cohort no social security number could be obtained suggesting possible under-estimation of deaths.

Comparison of high- with low-exposure groups revealed significantly lower mortality from life-style associated causes of death (lung cancer, vascular diseases, diabetes mellitus, chronic obstructive pulmonary disease, and liver cirrhosis) and significantly higher mortality from all leukemias and external causes of death. Increased mortality from leukemias was found in all high exposure groups but the most pronounced increase was observed in aviation electronics technicians. Brain cancer was less frequent in all high exposure groups compared to the low exposure category.

The long period of follow up of this large cohort with start of follow up almost at the same time (1950-54) and at a time when exposure commenced is a great advantage of this investigation. However, there are a number of shortcomings: follow up was possibly incomplete by unknown social security number of a substantial proportion of the cohort; almost half of all deaths in the first 20 years were from external causes which could have obscured an effect of exposure; duration and intensity of exposure is unknown as well as potential exposure after leaving the Navy; classification into low and high exposure groups may introduce substantial misclassification. In the earlier report, inspection of Navy records for a sample from the high exposure group revealed that 24% had no exposure to radar waves at all.

Concerning brain tumors, assuming an effect of radar exposure on tumor growth rate, exposure during the Korean War and no exposure afterwards would be expected to result in only a slightly increased risk during a period of about 10 years after the war. Sailors were about 20 to 25 years at that time. The fraction with an already initiated brain tumor during this age range is estimated to be less than 3 in 100,000 per year. Increase of growth rate even if substantial cannot result in an effect observable in a cohort of that size. If radar exposure increases the likelihood of malignant transformation this could increase the incidence during a time window of 10 to 30 years after the exposure period. Results of the Israeli study of x-ray treated tinea capitis (Sadetzki et al. 2005) suggests an average latency of about 20-25 years, however, risk decreased with increasing age at first exposure to x-rays. Taking the data on ionizing radiation as a guiding principle for brain tumor initiation, radar exposure of sailors during their twenties might result in an increase of brain tumor mortality of about 10 to 15%, i.e. a maximum of 8 additional cases among 20,000. Considering the biases of the study such a low risk is easily obscured. Hence neither tumor promotion nor initiation may be detected in this study even if there is an increased risk. Because of the mentioned limitation to a certain time window with possibly increased incidence due to exposures during service in the Korean War, it would have been instructive to compute Kaplan-Meier estimates for cumulative brain tumor mortality.

O. Ha et al. 2002

An ecological study around 11 high-power AM transmitter study sites (i.e., 100–1,500-kW transmission power) and 31 low-power study sites (i.e., 50-kW transmission power) used for comparison was conducted in South Korea. For each high-power site four control areas located in the same or nearest adjacent province as the high-power site, but were at least 2 km from any of the transmitters were chosen. The incidence of cancer within a 2-km radius of each transmitter and within control districts was obtained from Korean medical-insurance records for the years 1993 through 1996. Standardized incidence ratios (SIR) of high- against low-power transmitter areas were reported and additionally observed-to-expected ratios for each type of transmitter. SIRs were elevated for all cancers and for female brain cancer. Concerning transmitter types, for all types except 250 kW elevated OER for brain cancer were obtained (statistically significant for 100 kW).

Due to the complex relationship between distance and field strength, depending on antenna type and characteristics, height above ground level, orographic conditions, electrical properties of the terrain, etc., choice of a 2-km radius for all transmitters might not have been the best option to select the highest exposure group.

P. Park et al. 2004

A similar design as in the study of Ha et al. (2003) was applied in this ecological investigation of cancer deaths. Ten high-power (i.e., 100–1,500-kW transmission power) sites were chosen and compared to four control districts as in the previous study. Standardized mortality ratios were elevated for all single cancer sites but significant only for total cancer deaths. For brain cancer the ratio was 1.52 and statistically not significant.

The same criticism as for the study of Ha et al. (2003) applies to this study. Both studies share the limitations inherent in the ecological study design.

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Q. Berg et al. 2006

In the German part of the Interphone study special attention was paid to occupational history and exposure to RF fields at workplaces. Incident meningioma (n=381, response rate 88%) and glioma cases (n=366, response rate 80%) aged 30-69 years were selected from four neurological clinics. Overall 1,535 (participation rate 63%) were randomly selected from population registries matched to the cases by sex, age, and region. Most cases were interviewed during their stay in hospitals, controls were interviewed at home. The interview contained several screening questions about occupations that are probably associated with RF exposure. If any of these screening questions were marked additional questions were asked about the job. Based on the literature and the evaluation by two industrial hygienists a classification into the following categories was performed: no RF exposure/not probably RF exposed/probably RF exposed/highly RF exposed. In total about 13% (299 cases and controls) were classified with at least possible RF exposure at the workplace. Analyses were adjusted for region, sex, age, SES, urban/rural residence, ionizing radiation exposure in the head/neck region. Mobile phone use was not considered as a confounder.

While overall RF exposure at workplaces showed no increased odds-ratios, high exposure and especially for durations of 10 years or more resulted in elevated risk estimates that were, however, not significant. This result was similar for meningioma (OR=1.55 for high exposure for 10 years or more) and glioma (OR=1.39).

The study tried to assess potential workplace exposure as precisely as possible in a personal interview, but still misclassification may have occurred especially in the probable and not probable categories while the high exposure group is likely to have had at least occasionally above average RF exposure. Odds ratios are in the range expected if exposure results in a substantial increase of growth rate. The small number of highly and long-term exposed cases (13 glioma and 6 meningioma) prohibit, however, far reaching conclusions.

R. Schüz et al. 2006

In the same study as mentioned above also exposure to emissions from DECT (Digital Enhanced Cordless Telecommunications) base stations near the bed were analyzed. Both, for glioma and meningioma, not significantly decreased odds ratio were reported. There was also no increasing risk observed with duration of exposure to DECT cordless phone base stations. The study was limited due to the small number of exposed subjects and the short exposure duration. It is unlikely that after these short exposures periods an increased risk can be observed.

S. Hu et al. 2007

The study from South Korea that was a major improvement in investigating the possible association between RF EMF exposure and cancer risk applied not only instead of an ecological approach the case-control paradigm but also used an interesting method to estimate individual exposure. This method seems a reasonable compromise between effort and precision. The study included leukemia and brain cancer patients under age 15 years and controls with respiratory illnesses matched to cases on age, sex, and year of diagnosis (1993–1999). All were selected from 14 South Korean hospitals using the South Korean Medical Insurance Data System. Residential addresses were obtained from medical records so that no direct contact with the participants was necessary. Authors developed an exposure prediction program incorporating a geographic information system that was modified by the results of actual measurements carried out systematically at defined locations and during driving along specific trajectories. Furthermore, electrical characteristics of the environment were considered. This method was used to estimate RF EMF exposure from 31 AM radio transmitters with a power of 20 kW or more. A total of 1,928 leukemia patients, 956 brain cancer patients, and 3,082 controls were included.

A significantly increased odds ratio was obtained for childhood leukemia at a distance of 2 km or less from the transmitters relative to a distance of >20 km. In response to a critical comment by Schüz et al. (2008) authors recalculated the risk estimates for total and peak RF EMF exposure (Hu et al. 2008) and reported for the highest quartile of peak RF EMF exposure a significantly increased risk of ALL. For childhood brain cancers insignificantly increased risks of about 1.4 for ≤2 km and 2-4 km from the transmitter were obtained.

It seems that there were problems with the RF EMF estimates since peak and total field strengths had quite different results and also the correlation with peak exposure and distance was much higher than with total exposure suggesting that more distant transmitters led to a decrease in the gradient of exposures. The measurements are not reported for the different transmitter types and therefore it is difficult to assess their validity. For very high power transmitters (1,500 kW) the relationship is known to be not monotonous which cannot be discriminated in the figure shown in the article. Overall the study has an improved methodology due to the case-control and registry approach. However, the methods to assess actual exposure need to be further improved.

IV. EVALUATION OF THE EVIDENCE

Due to the varying endpoints, methods used and populations included the meta-analysis shown in fig.1 applied the random effects model and DerSimonian-Laird estimate of the overall risk and confidence interval. Only few studies found clear indications of an association between RF exposure and brain tumors: one cohort study (Szmigielski 1996) and two case-control studies (Thomas et al. 1987, Grayson 1996). None of the ecological studies except for Ha et al. (2003) for one of the AM transmitter types demonstrated a significantly increased risk in the vicinity of RF antennas.

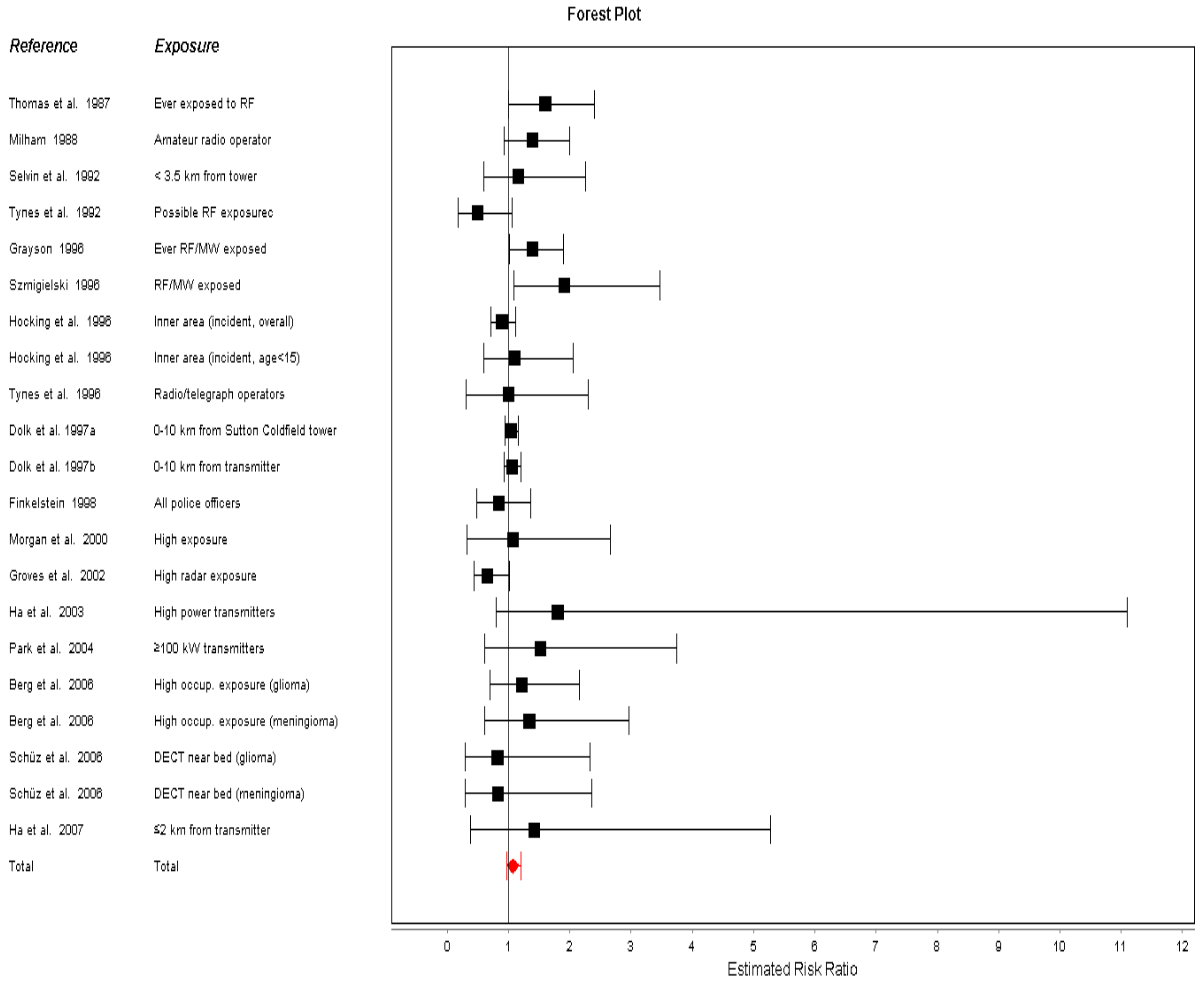

Fig. 1: Forest plot of risk estimates for RF exposure with respect to brain tumors and DerSimonian-Laird overall estimate

The meta-analytical estimate of the risk was 1.08 (95% confidence interval: 0.97 – 1.20). The discussion of the 19 published investigations revealed shortcomings in all studies. The greatest problem was encountered in the difficulties to reliably assess actual exposure. Even if we don't know the relevant aspect of the exposure, if any, that is responsible for an increased risk, the type, duration and amount of exposure must be determined in order to use the studies in derivations of exposure standards. None of the studies included a useful quantitative indicator of intensity of exposure and even duration of exposure was rarely addressed. Concerning type of exposure only quite crude and broad categories were used.

In ecological studies, although for the studied population the exposure - despite considerable variations in time - is similar with respect to carrier frequency, modulation etc. it is quite different between various types of transmitters and hence results are not easily generalized. The ecological studies are not conclusive with respect to brain tumors but provide some evidence for hematopoietic malignancies that need to be further pursued. Investigating residential exposure to RF EMFs from broadcasting stations poses severe methodological problems mainly due to the small size of the exposed population because high exposure levels occur only in a small band around the radiation sources. Due to the transition to digital television many TV broadcasting antennas with high power are or will be disconnected leaving us with changing exposure conditions. Because brain tumors have long latencies it is hardly possible to produce conclusive evidence in the near future.

Considering the discussion of the different investigations and the fact that most biases encountered tend to dilute a potential risk, the compiled evidence from occupational cohorts is compatible with a moderately increased risk of RF exposure. Because of the lack of actual measurements but observing that exposure above guideline levels must have been a rare event a precautionary approach must result in a reduction of occupational exposure levels and organizational measures to avoid over-exposure and also environmental exposure levels should be given greater attention. Although brain tumors are rare and the population attributable risk is low (assuming 13% of adults being occupationally exposed to RF fields as inferred from Berg et al. 2006, and assuming a relative risk of 1.3, about 4% of brain tumors can be attributed to RF exposure, i.e. 2,200 cases per years in the US).

CONCLUSIONS

- Only few studies of long-term exposure to low levels of RF fields and brain tumors exist, all of which have methodological shortcomings including lack of quantitative exposure assessment. Given the crude exposure categories and the likelihood of a bias towards the null hypothesis of no association the body of evidence is consistent with a moderately elevated risk.

- Occupational studies indicate that long term exposure at workplaces may be associated with an elevated brain tumor risk.

- Although in some occupations and especially in military jobs current exposure guidelines may have sometimes been reached or exceeded, overall the evidence suggest that long-term exposure to levels generally lying below current guideline levels still carry the risk of increasing the incidence of brain tumors.

- Although the population attributable risk is low (likely below 4%), still more than 2,000 cases per year in the US can be attributed to RF exposure at workplaces alone. Due to the lack of conclusive studies of environmental RF exposure and brain tumors the potential of these exposures to increase the risk cannot be estimated. However, these figures are theoretical as long as the evidence is as weak as it is for the time being.

V. ASSESSMENT OF EPIDEMIOLOGICAL EVIDENCE BY IEEE (C95.1 REVISION)

Introduction

Before 1988 C95 standards were developed by Accredited Standards Committee C95, between 1988 and 1990, the committee was converted to Standards Coordinating Committee 28 (SCC 28) under the sponsorship of the IEEE Standards Board. In 2001 IEEE approved the name "International Committee on Electromagnetic Safety (ICES)" for SCC 28. Subcommittee 4 of ICES Technical Committee 95 is responsible for the revision of standard C95.1 "IEEE Standard for Safety Levels with Respect to Human Exposure to Radio Frequency Electromagnetic Fields, 3 kHz to 300 GHz". There are five TC95 subcommittees: 1) Techniques, Procedures, and Instrumentation; 2) Terminology, Units of Measurements and Hazard Communication; 3) Safety Levels with Respect to Human Exposure, 0-3 kHz; 4) Safety Levels with Respect to Human Exposure, 3 kHz-300 GHz; 5) Safety Levels with Respect to Electro-Explosive Devices.

The recommendations in standard C95.1 are intended to protect against scientifically established adverse health effects in human beings resulting from exposure to radio frequency electromagnetic fields in the frequency range of 3 kHz to 300 GHz. A "scientifically established adverse health effects" is defined as: "A biological effect characterized by a harmful change in health that is supported by consistent findings of that effect in studies published in the peer-reviewed scientific literature, with evidence of the effect being demonstrated by independent laboratories, and where there is consensus in the scientific community that the effect occurs for the specified exposure conditions." It is interesting that this definition does not only demand the effect being demonstrated by independent laboratories but also that a consensus must be reached in the scientific community. This is a strange definition. When is a consensus reached? If more than 50% of scientists in the scientific community agree? Or must all agree? Usually this term is used to describe a situation where there is no open or covert dissent. In decisions theory demanding consent is criticized as a policy that results in the preservation of the status-quo.

It might be instructive to contrast this definition with IARCs (International Agency for Research on Cancer) characterization of sufficient evidence for carcinogenicity in experimental animals: "The Working Group considers that a causal relationship has been established between the agent or mixture and an increased incidence of malignant neoplasms or of an appropriate combination of benign and malignant neoplasms in (a) two or more species of animals or (b) in two or more independent studies in one species carried out at different times or in different laboratories or under different protocols", and the characterization of sufficient evidence in humans: "The Working Group considers that a causal relationship has been established between exposure to the agent, mixture or exposure circumstance and human cancer. That is, a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence." Clearly these definitions are incompatible with the definition by IEEE.

The scientific rationale for the derivation of the exposure standard of IEEE is presented in Annex C and Annex B "Identification of levels of RF exposure responsible for adverse effects: summary of the literature" which is based on "critical reviews of studies within the IEEE/WHO RF literature database". In this commentary I will address chapter 9) Epidemiological Studies of RF Exposures and Human Cancer.

Evaluation of Cancer-Related Endpoints (RF Exposure)

In their 2006 revision of the standard C95.1 IEEE has assessed the evidence from epidemiology for cancer related endpoints in chapter B.7.3. The assessment relies mainly on the reviews of Bergqvist (1997), Moulder et al. (1999) and Elwood (2003). These reviews and the IEEE overview share the same deficiencies. The main lines of argumentation would be impossible in any other field of environmental health and closely resemble the strategy used to dismiss a power frequency exposure/childhood leukemia association. In the following paragraphs the assessment by IEEE will be discussed. The text of IEEE C95.1 is presented in italics as blocked citation. References within the text of the citations are found by the Rnnn and Bnnn numbers in the Annexes F and G of the standard document, but are also included in the reference section of this overview.

> *Cluster studies, such as the one performed in Sutton Coldfield in the U.K. in response to a cluster of leukemia and lymphoma in adults living close to an RF broadcasting transmitter (Dolk et al. [R624]), are inherently difficult to interpret because of the impossibility of assessing all of the effects that chance variation might have contributed to the cluster. In the initial Sutton Coldfield study, the authors correctly concluded that no causal association could be drawn between the presence of the cluster and RF exposure from broadcasting towers (Dolk et al. [R625]) (Cooper et al. [R760]). (IEEE C 95.1 – 2005, p.75)*

First of all the Sutton Coldfield study was no cluster study but an ecological investigation. It only was initiated by an unconfirmed report of a cluster of leukemia and lymphoma in the vicinity of this broadcasting transmitter but it proceeded independently of this initial report and used registry data of the population living within a radius of 10 km around the transmitter. The statement that such studies are "inherently difficult to interpret because of the impossibility of assessing all of the effects that chance variation might have contributed to the cluster" is ridiculous not only because the study is no cluster study but because it is impossible for any study to "assess all effects that chance variation might have contributed" to the endpoint under investigation. It is not mentioned that the study was supplemented by a larger investigation of another 20 high-power transmitters in Great Britain. The difficulties of interpreting ecological studies is related to the fact that potential confounders can only be related to a segment of the population but not to individuals and that in general duration and intensity of exposure are not known for individual members of the different strata. While evidence for an effect on brain tumor incidence from both studies (Dolk et al. 1997a, 1997b) is weak, there is consistent evidence for a relation to hematopoietic cancers. This evidence has been overlooked by the authors due to their wrong assumption about the relation between proximity to the transmitter and exposure.

> *Inconsistent effects have been reported between residential proximity to other RF broadcast towers and adverse health endpoints (Bielski [R267]) (Maskarinec et al. [R579]) (Selvin and Merrill [R823]) (Michelozzi et al. [R858]) (Altpeter et al. [R977]) (Hallberg and Johansson [R995], [R996]) (Boscolo [R1012]), although many of these studies have significant flaws in their study design (making them difficult to interpret). (IEEE C 95.1 – 2005, p.75)*

Although it is not stated what these "inconsistent effects" might be, the statement is flawed in more than this respect. First of all the study by Bielski (1994) is an occupational investigation and not about residential proximity to RF broadcast towers, second three of these investigations (Selvin et al. 1992; Maskarinec et al. 1994; Michelozzi et al. 2002) included leukemia as an endpoint with indications of an increased incidence consistent with the studies from Great Britain (Dolk et al. 1997a, 1997b) and Australia (Hocking et al. 1996). Note that the study by Selvin et al. (1992), as stated in section 10, intended to compare different methods to assess the relationship between a point source and diseases and did erroneously assume a monotonous relationship between exposure and distance from a transmitter. Correcting this error there seems to be an increased probability of childhood leukemia in areas receiving the highest exposure from the Sutro tower. The other three investigations (Altpeter et al. 1995; Boscolo 2001; Hallberg & Johansson 2002) have nothing in common and hence cannot be inconsistent.

> *An increased incidence and mortality rate of childhood leukemia was reported in Australia with residential proximity to a specific RF broadcasting tower (Hocking et al. [R633]), although subsequent reanalysis of the data showed the results may have been influenced by other confounding variables within the study location (McKenzie et al. [R669]). (IEEE C 95.1 – 2005, p.75)*

This is another example how carelessly and sloppy the evidence is dealt with by the IEEE committee. The study of Hocking et al. (1996) was not about "proximity to a specific RF broadcasting tower" but about an area where three broadcasting towers are located. While there is always the possibility of confounders influencing results of an epidemiologic investigation, the 'reanalysis' of McKenzie et al. (1998) is seriously flawed and cannot support the cited statement. Hocking et al. (1996) combined the districts near the broadcasting area and those further away based on homogeneity analyses, while McKenzie et al. (1998) omitted one area with high incidence (and highest exposure) based on inspection of data. Any statistical analysis subsequent to such data picking is useless.

> *While scattered reports of adverse health effects associated with occupational exposure to RF do exist (Demers et al. [R36]) (Kurt and Milham [R68]) (Pearce [R110]) (Speers et al. [R125]) (Thomas et al. [R128]) (Pearce et al. [R199], [R211]) (Hayes et al. [R207]) (Cantor et al. [R268]) (Davis and Mostofi [R563]) (Tynes et al. [R570], [R605]) (Grayson [R592]) (Richter et al. [R747]) (Holly et al. [R838]) these studies are largely inconsistent with each other in terms of the adverse health endpoints affected, and often show no clear dose response with RF exposure. Many have serious flaws in their study design, contain limited or insufficient RF exposure assessment, and are generally inconsistent with the absence of findings of an association from other occupational studies (Tornqvist et al. [R131]) (Coleman [R142]) (Lilienfeld et al. [R146]) (Robinette and Silverman [R147], [R148]) (Siekierzynski et al. [R151], [R152]) (Wright et al. [R213]) (Coleman et al. [R214]) (Muhm [R506]) (Czerski et al. [R542]) (Hill [R568]) (Lagorio et al. [R616]) (Kaplan et al. [R647]) (Morgan et al. [R701]) (Gallagher et al. [R822]) (Groves et al. [R853]) (Wiklund [R1013]) (Armstrong et al. [R1014]). (IEEE C 95.1 – 2005, p.75)*

Even allowing for restrictions of space for a discussion of the evidence, greater nonsense has not been produced so far in this field as condensed in these two sentences. Putting higgledy-piggledy all sorts of studies together and then wondering about endpoints being inconsistent is an intellectual masterpiece. Of the occupational studies mentioned, three (Thomas et al. 1987; Speers et al. 1988; Grayson 1996) were about brain cancer, three about hematopoietic cancers

(Pearce et al. 1985; Kurt & Milham 1988; Pearce 1988), two about testicular cancer (Hayes et al. 1990; Davis & Mostofi 1993), one about male (Demers et al. 1991) and two about female breast cancer (Cantor et al. 1995, Tynes et al. 1996) the latter including other cancers as well, and one about intraocular melanoma (Holly et al. 1996). Three further studies (Pearce et al. 1989; Tynes et al. 1992; Richter et al. 2000) investigated several or all malignancies. These studies differ not only in endpoints, study type (cohort, case-control, and cluster) but also in the methods of exposure assessment. Ignorance of the IEEE reviewers is underlined by the compilation of studies characterized by an “absence of findings of an association”. Not only did several of these studies indeed indicate an association of cancer risk with EMF exposure (Lilienfeld et al. 1978; Robinette et al. 1980; Tornqvist et al. 1991; Armstrong et al. 1994; Lagorio et al. 1997; Groves et al. 2002) but two were no epidemiologic studies at all (Siekierzynski et al. 1974; Czerski et al. 1974) and several were rather addressing ELF exposure (Tornqvist et al. 1991; Wright et al. 1982; Coleman et al. 1983; Gallagher et al. 1991) and one (Wiklund 1981) was a cluster study in the telecommunication administration with uncertain type of exposure. Simply confronting studies finding an effect with others that were ‘negative’ is scientifically flawed and permits neither the conclusion that there is nor that there is no association between exposure and cancer risk. Even if all studies would have applied the same method, assessed the same endpoint and used the same exposure metric, studies reporting a significantly increased cancer risk are not outweighed by others that did not.

> *While micronuclei formation in workers occupationally exposed from broadcast antennas has been reported (Garaj-Vrhovac [R757]) (Lalic et al. [R791]), these findings were not verified in a larger study of more than 40 Australian linemen exposed under similar conditions (Garson et al. [R186]). (IEEE C 95.1 – 2005, pp.75-76)*

It goes without saying that also this statement is wrong. Garson et al. (1991) did not investigate micronuclei formation, their workers were considerably shorter exposed and it were not more than 40 linemen but 38 radio-lineman.

> *No clear association could be established between occupational exposures of parents to a number of agents, including RF, and effects (neuroblastoma) in their offspring (Spitz and Johnson [R289]) (De Roos et al. [R798]). (IEEE C 95.1 – 2005, p.76)*

What is meant by ‘no clear association’ is obscure. Spitz and Johnson (1985) found a significantly increased risk after paternal occupational exposure to electromagnetic fields, and also De Roos et al. (2001) found several jobs with paternal as well as maternal exposure to

EMFs associated with an elevated risk for neuroblastoma in their children. However, broad groupings of occupations with ELF, RF EMF, as well as ionizing radiation (!) exposure did not reveal an increased risk.

> *One study reported a slight excess in brain tumors associated with combined exposure to RF and other exposures associated with electrical or electronic jobs, but not with RF alone (Thomas et al. [R128]). A study of a Polish military cohort reported a substantial excess of total cancer and several cancer sub-types with jobs associated with RF exposure (Szmigielski [R578]), (Szmigielski and Kubacki [R982]), although questions have been raised about severe bias in the exposure assessment of this study (Elwood [R665]) (Bergqvist [R1015]) (Stewart [R1133]). Studies by Milham of U.S. amateur radio operators reported an excess in one of nine types of leukemia assessed (see [R101], [R102], [R209], [R215], and [R569]), but not for total tumors, total leukemia, or brain tumors, and potential confounding factors might have included exposure to soldering fumes, degreasing agents and over-representation of a particular social class. (IEEE C 95.1 – 2005, p.76)*

Again the evidence is incorrectly summarized for all cited investigations. Thomas et al. (1987) found a significantly elevated risk for brain tumors among all men exposed to RF fields and in particular in those exposed for 20 or more years. There were indications that this elevated risk is due to a subgroup with electrical or electronics jobs. The group of those exposed in other jobs is heterogeneous and may contain subjects with low or no exposure (e.g. some groups of welders) and therefore lack of an association could be due to a dilution effect from exposure misclassification.

As mentioned in section 10 criticism of the Polish military cohort study about exposure assessment is unfounded. Bergqvist (1997), Elwood (1999) and Stewart (2000) criticized that the military health board assessed a number of potential risk factors only for cancer cases. However, they overlooked that the study was a cohort and not a case-control study and that at no stage information about these factors entered the analysis and therefore couldn’t affect the results in any way.

The study by Milham (1988a, 1988b) of radio amateur operators revealed a significantly increased standardized mortality ratio (SMR) for acute myeloid leukemia while the overall mortality and cancer mortality was significantly reduced relative to the country mortality rates. As mentioned in section 10 this points to a ‘healthy worker’ effect as well as to an influence of life-style factors (mortality related to smoking and overweight were reduced). From the mentioned nine types of leukemia three with expectancies below one and no case observed couldn’t be assessed, from the six remaining types five had elevated SMRs with AML, the most frequent type in adults, being significantly elevated.

The last portion of the IEEE review of epidemiology studies is dedicated to mobile phone investigations that are discussed in another contribution.

The following citation presents the IEEE summary in its full length:

> *The epidemiological evidence to date does not show clear or consistent evidence to indicate a causal role of RF exposures in connection with human cancer or other disease endpoints. Many of the relevant studies, however, are weak in terms of their design, their lack of detailed exposure assessment, and have potential biases in the data. While the available results do not indicate a strong causal association, they cannot establish the absence of a hazard. They do indicate that for commonly encountered RF exposures, any health effects, if they exist, must be small. Even though epidemiological evidence cannot rule out a causal relationship, the overall weight-of-evidence is consistent with the results of the long term animal studies showing no evidence of physiological, pathological or disease-specific effects. (IEEE C95.1 - 2005; pp.76-77)*

As already pointed out earlier (Kundi 2006) there is an intolerable tendency in the past years that confronted with an undeniable epidemiologic evidence of an association between an agent and adverse health effects such as cancer, interested parties take their resort to the concept of causality based on the wrong assumption evidence to “indicate a causal role” is a lot more difficult to provide. Unprecedented, however, is the notion of “a strong causal association”. Whatever the meaning of this exceptional statement, the conclusion that, if health effects of commonly encountered RF exposures exist, they must be small, is wrong. To the contrary: considering the “lack of detailed exposure assessment” and other potential biases that predominantly lead to an underestimation of the risk, the evidence points to a quite substantial risk. While the animal studies reviewed in another section of the IEEE standard document cannot be discussed here it should be underlined that they are generally insufficient to support either an increased risk or the lack of health relevant effects. Therefore they cannot be used in a weight-of-evidence statement as has been made by IEEE, that there is no evidence for adverse health effects of RF exposure.

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SECTION 12

Evidence for Childhood Cancers (Leukemia)

2012 Supplement

(Replaces 2007 Chapter)

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I. INTRODUCTION

The International Agency for Research on Cancer (IARC) concluded in 2001 that power-frequency magnetic fields are a possible human carcinogen (Group 2B). This classification was based on the evidence from epidemiological studies of childhood leukemia. The panel rated the evidence from all other types of cancer, from long-term animal experiments and mechanistic studies as inadequate. The IARC working group decided that the association between power frequency magnetic fields and childhood leukemia can be interpreted as only limited evidence because bias and confounding cannot be ruled out.

Since the seminal work of Wertheimer and Leeper (1979) many epidemiological studies of childhood cancer and residential exposure to power-frequency EMFs were published, not counting some studies about electrical appliances and cluster observations. Although these studies make up an impressive body of evidence, there is an ongoing discussion whether the observed relationships between exposure to power-frequency EMFs and childhood cancer (in particular leukemia) can be causally interpreted. Based on the comparatively few empirical studies virtually hundreds of commentaries, reviews and meta-analyses have been produced, more often than not increasing confusion instead of clarifying the issue. In 2000 two pooled analyses of childhood leukemia, the endpoint most often studied, have been published, one (Ahlbom et al., 2000) that was restricted to 9 studies that fulfilled a number of strict inclusion criteria (a defined population base for case ascertainment and control selection and using measurements or historical magnetic field calculations for exposure assessment), and another (Greenland et al., 2000) including also wire-code studies. Both pooled analyses got essentially the same result: a monotonously increasing risk with increasing power-frequency (50Hz/60Hz) magnetic field levels. These pooled analyses were the bases for the IARC working group decision.

Typically, if an agent is classified as a Group 2B carcinogen, precautionary measures are taken at workplaces and special care is recommended if it is present in consumer products (e.g. lead, styrene, benzofuran, welding fumes). Concerning power-frequency EMFs the WHO International EMF Program made the following exceptional statement: “In spite of the large number data base, some uncertainty remains as to whether magnetic field exposure or some other factor(s) might have accounted for the increased leukaemia incidence.” (WHO Fact Sheet 263, 2001). This is the line of arguments that has been unswervingly followed by the electrical power industry since the early 1980’s. An endless chain of factors allegedly responsible for the ‘spurious’ positive association between power-frequency EMF exposure and cancer has been put forward, leading to nothing except waste of energy and money. The statement of WHO is scientifically flawed because there is no finite number of empirical tests to refute it. It is always possible that some factor not yet tested could be responsible, however low the probability that it remained obscure for such a long time. In the last years, due to the fact that no confounding factor has been found that explains the increased leukemia risk, a slight change of arguments can be discerned that consists of pointing out the very low proportion of children (less than 1%) exposed to power frequency fields associated with a significantly increased risk. In fact, both pooled analyses concluded that there is little indication of an increased risk below 3 to 4 mG magnetic flux density.

Since the evaluation of IARC several other epidemiological studies have been published that corroborate the earlier findings and strengthen the evidence of an association. It becomes increasingly less likely that confounding factors exist that operate all over the world and still remained undetected.

In the following chapters we will present the epidemiological evidence, discuss potential biases and demonstrate that from a worst-case scenario the evidence compiled so far is consistent with the assumption of a much greater proportion of leukemia cases attributable to power frequency field exposure than previously assumed. The key problem identified is the lack of a bio-physical model of interaction between very weak ELF EMFs and the organism, tissues, cells, and biomolecules.

II. EPIDEMIOLOGICAL STUDIES OF POWER-FREQUENCY EMF AND CHILDHOOD CANCER

Table 11-4 gives a synopsis of studies on childhood cancer and exposure to power-frequency EMF, Table 11-5 presents the main findings of these investigations. Most often assessment of exposure was by measurements with 16 studies measuring for at least 24 hours up to 7 days, and 9 studies with spot measurements. Eleven studies used distance from power lines as a proxy (some in combination with spot measurements) and 11 studies used wire codes (solely or in addition to other methods) classified according to the Wertheimer-Leeper or Kaune-Savitz methods or some modifications thereof accounting for specific power grid conditions. Several investigations covered more than one endpoint with hematopoietic cancers the most frequently included malignancies (overall 37 studies), followed by nervous system tumors (13 studies) and other cancers (10 studies). All childhood cancer cases were assessed by 9 investigations.

The most restrictive criteria for combining the evidence for an association between ELF magnetic fields (MF) exposure and childhood leukemia were applied by Ahlbom et al., (2000) that included 9 investigations. Table 11-1 shows the results of these investigations for the exposure category ≥ 4 mG (against < 1 mG as reference category). The studies included 3,203 children with leukemia, 44 of which were exposed to average flux densities of 4 mG or above. Thus only 1.4% of children with leukemia and less than 1% of all children in the studies were exposed that high in accordance with measurement samples from the general population in Europe, Asia and America (Brix et al., 2001; Decat et al., 2005; Yang et al., 2004; Tomitsch et al. 2010; Zaffanella, 1993; Zaffanella & Kalton, 1998).

Meta-analyses of wire-code studies (Greenland et al., 2000; Greenland 2003; Wartenberg, 2001) revealed similar results for childhood leukemia with estimates of risks around 2 for very high current codes but with considerable heterogeneity across studies.

Table 11- 1: Results from nine studies included in Ahlbom et al. (2000) updated according to Schüz (2007) of residential MF exposure and risk of childhood leukemia

Country	Odds-Ratio*) (95%-CI)	Observed Cases
Canada	1.55 (0.65−3.68)	13
USA	3.44 (1.24−9.54)	17
UK	1.00 (0.30−3.37)	4
Norway	0 cases / 10 controls	0
Germany	3.53 (1.01−12.3)	7
Sweden	3.74 (1.23−11.4)	5
Finland	6.21 (0.68−56.9)	1
Denmark	2 cases / 0 controls	2
New Zealand	0 cases / 0 controls	0
Overall	2.08 (1.30 – 3.33)	49

*) 24-h geometric mean MF flux density of ≥ 4 mG against <1 mG

In 2010 Kheifets et al. published a pooled analysis of studies that appeared after the analyses of Ahlbom et al. (2000) and Greenland et al. (2000). This analysis included data from Bianchi et al. (2000) , Kabuto et al. (2006), Kroll et al. (2010), Lowenthal et al. (2007), Malagoli et al. (2010), Schüz et al. (2001), and Wünsch-Filho et al. (2011). For this pooled analysis the data from Bianchi et al. (2000) were extended by 5 years. Table 11-2 gives an overview of the results of this pooled analysis.

Table 11- 2: Results from the pooled analysis of 7 (6) studies of residential MF exposure and risk of childhood leukemia (Kheifets et al. 2010a) and of the earlier pooled analysis of 9 other studies (Ahlbom et al. 2000). Shown are odds ratios (95% confidence interval) adjusted for age, sex, SES and study.

Exposure category	Kheifets et al. 2010a	Kheifets et al. 2010a without Brazil	Ahlbom et al. 2000
<1 mG (ref)			
1-2 mG	1.07 (0.81 – 1.41)	1.15 (0.83 – 1.61)	1.08 (0.89 – 1.31)
2-4 mG	1.22 (0.78 – 1.89)	1.20 (0.67 – 2.17)	1.11 (0.84 – 1.47)
≥4 mG	1.46 (0.80 – 2.68)	2.02 (0.87 – 4.69)	2.00 (1.27 – 3.13)
>200 m (ref)			
100-200 m	1.20 (0.90, 1.59)		
50-100 m	1.30 (0.89, 1.91)		
≤50 m	1.59 (1.02, 2.50)		

In addition to studies investigating the risk of leukemia in relation to power frequency MF the hypothesis has been examined that effects on relapse and survival in newly diagnosed acute lymphoblastic leukemia occur (Foliart et al. 2006, 2007). There was a significantly increased hazard ratio for death at exposures ≥3 mG that was based on four deaths only.

The only other endpoint except leukemia and other hematopoietic diseases that has been investigated in several studies is nervous system tumors. The number of cases studied is too low to allow a differentiation according to diagnostic subgroups. Several papers have investigated childhood CNS tumors amongst other endpoints, including leukemia (Wertheimer & Leeper, 1979; Tomenius, 1986; Savitz et al., 1988; Feychting & Ahlbom, 1993; Olsen et al., 1993; Verkasalo et al., 1993; Tynes & Haldorsen, 1997; UKCCS, 1999; 2000; Draper et al., 2005; Kroll et al., 2010), whereas others have solely investigated CNS tumors (Gurney et al., 1996; Preston-Martin et al., 1996; Schüz et al., 2001b; Saito et al., 2010). In most cases the time window was restricted to the postnatal period. Exposure was assessed based on residential proximity to overhead power lines, measurements and wiring configurations of houses. In a meta-analysis of childhood brain tumor studies (Wartenberg et al., 1998) estimates of risk were similar whether based on calculated fields (OR 1.4, 95% CI: 0.8 – 2.3), measured fields (OR 1.4, 95% CI: 0.8 – 2.4), wire codes (OR 1.2, 95% CI: 0.7 – 2.2), or proximity to electrical installations (OR 1.1, 95% CI: 0.7 – 1.7). The few studies published after this review do not change these figures substantially. Kheifets et al. (2010) report a pooled analysis of 10 studies using measured or calculated fields. The results are summarized in Table 11-3.

Table 11- 3: Summary of results from a pooled analysis of 10 studies of residential MF exposure and risk of childhood brain tumors (Kheifets et al. 2010b). Shown are odds ratios (95% confidence interval) adjusted for age and sex.

		Type of measurement	
Exposure category	Long-term	Calculated fields	Spot
<1 mG (ref)			
1-2 mG	1.13 (0.69 - 1.87)	1.06 (0.53 - 2.11)	1.16 (0.79 - 1.72)
2-4 mG	0.94 (0.43 - 2.06)	0.56 (0.19 - 1.60)	1.21 (0.67 - 2.18)
≥4 mG	1.35 (0.39 - 3.71)	1.21 (0.53 - 2.78)	0.68 (0.26 - 1.80)
		Type of home exposure	
Exposure category	Home at diagnosis	Longest lived-in	Birth home
<1 mG (ref)			
1-2 mG	0.89 (0.60 - 1.31)	1.42 (0.79 - 2.56)	1.03 (0.59 - 1.80)
2-4 mG	0.77 (0.44 - 1.36)	0.86 (0.28 - 2.65)	0.79 (0.34 - 1.80)
≥4 mG	1.08 (0.54 - 2.16)	2.19 (0.57 - 8.44)	1.14 (0.52 - 2.49)

III. DISCUSSION

With overall 42 epidemiological studies published to date power frequency EMFs are among the most comprehensively studied environmental factors. Except ionizing radiation no other environmental factor has been as firmly established to increase the risk of childhood leukemia, but for both there are ongoing controversies. Although data from atomic bomb survivors and radiotherapy of benign diseases (ringworm, ankylosing spondylitis, and thymus enlargement) clearly indicate a causal relationship between exposure and leukemia, for other conditions like living in the vicinity of nuclear power plants, diagnostic x-rays, exposure secondary to the Chernobyl incident evidence is less clear and therefore no agreement has been reached so far. Concerning power frequency EMFs few deny that the relationship is real and not due to chance, but still there is a discussion whether or not this association can be causally interpreted. Still the possibility that confounding, exposure misclassification, and selection and other biases are responsible for the observed relationship is mentioned as an argument against a causal interpretation. Furthermore, it is often claimed that even if the exposure is causally related, due to the low attributable fraction no expensive measures to reduce exposure are warranted.

The Environmental Health Criteria 238 (WHO 2007) summarizes:

> Scientific evidence suggesting that everyday, chronic low-intensity (above 0.3–0.4 μT) power-frequency magnetic field exposure poses a health risk is based on epidemiological studies demonstrating a consistent pattern of increased risk for childhood leukaemia. Uncertainties in the hazard assessment include the role that control selection bias and exposure misclassification might have on the observed relationship between magnetic fields and childhood leukaemia. In addition, virtually all of the laboratory evidence and the mechanistic evidence fail to support a relationship between low-level ELF magnetic fields and changes in biological function or disease status. Thus, on balance, the evidence is not strong enough to be considered causal, but sufficiently strong to remain a concern.
>
> Although a causal relationship between magnetic field exposure and childhood leukaemia has not been established, the possible public health impact has been calculated assuming causality in order to provide a potentially useful input into policy. However, these calculations are highly dependent on the exposure distributions and other assumptions, and are therefore very imprecise. Assuming that the association is causal, the number of cases of childhood leukaemia worldwide that might be attributable to exposure can be estimated to range from 100 to 2400 cases per year. However, this represents 0.2 to 4.9% of the total annual incidence of leukaemia cases, estimated to be 49 000 worldwide in 2000. Thus, in a global context, the impact on public health, if any, would be limited and uncertain. (pp.11-12)

Concerning preventive measures with respect to long-term effects it is stated:

> Implementing other suitable precautionary procedures to reduce exposure is reasonable and warranted. However, electric power brings obvious health, social and economic benefits, and precautionary approaches should not compromise these benefits. Furthermore, given both the weakness of the evidence for a link between exposure to ELF magnetic fields and childhood leukaemia, and the limited impact on public health if there is a link, the benefits of exposure reduction on health are unclear. Thus the costs of precautionary measures should be very low. (p.13)

The sequence of arguments is as follows:

- There are possible biases, exposure misclassification and confounding that could lead to spuriously increased risks
- There is no support from animal experiments and mechanistic studies for the association found in epidemiological investigations
- Therefore the association cannot be causal interpreted
- Even if the association is causal the number of attributable cases is low because of the small proportion of exposed children
- Therefore only low-cost precautionary measures are warranted.

In the following sections we will challenge these arguments.

A. The association between power frequency MF and childhood leukemia

After the pooled analyses of Ahlbom et al. (2000) and Greenland et al. (2000) were published several other epidemiological investigations were conducted that did not change the conclusions of an association between power frequency MF and childhood leukemia. Seven of these additional investigations were included in a pooled analysis by Kheifets et al. (2010a). Seven other studies were excluded for several reasons: because only distance to power lines was assessed, because data were not available in time etc. Overall the results of all studies taken together speak in favor of an association between exposure to power frequency MF and childhood leukemia (see Table 11-5).

B. Confounding

A confounder is a factor that is associated with the agent in question as well as with the disease. Hence a confounder must be a risk factor for the disease. Concerning childhood leukemia it was clear from the very beginning that any suggested confounder must be purely speculative since there is no established environmental risk factor except ionizing radiation. Even if a condition can be found that is strongly associated with exposure to power frequency fields, if it is not associated with childhood leukemia it cannot confound the relationship. In the homogenous case, i.e. the association between EMF exposure and the confounder does not depend on disease status, and the confounder - leukemia association is independent of exposure to power frequency EMFs, even a stronger assertion can be proven: power frequency EMF remains a risk factor if the risk associated with the confounder is smaller than that associated with power frequency EMFs. Equation (1) gives the bias-factor for the homogenous case and dichotomous exposure variables (that can, however, easily be extended to categorical or continuous exposure variables):

$$B_F = \frac{1 + \pi_F(\Psi_{AF}\Psi_{DF} - 1)}{[1 + \pi_F(\Psi_{AF} - 1)][1 + \pi_F(\Psi_{DF} - 1)]} \qquad (1)$$

(π_F is the prevalence of the confounder, Ψ_{DF} is the odds ratio for the confounder with respect to the disease, and Ψ_{AF} is the odds ratio of the agent in question with respect to the confounder). From this equation it is immediately clear that if either Ψ_{DF} or Ψ_{AF} or both are 1 there is no bias (i.e. the confounder is no risk factor for the disease and/or the agent in question is not associated with the confounder). This equation can be used to obtain limiting conditions for the odds ratio of the confounder given specific associations with power frequency fields. This has been done by Langholz (2001).

Langholz (2001) investigated factors that have been proposed as possible confounders based on data from Bracken et al. (1998). None of these factors on their own explain the power frequency EMF - leukemia relationship. It has been criticized (Greenland, 2003) that too far reaching conclusions have been drawn based on the failure to discover a single factor that may explain the relationship, because combinations of such factors have not been addressed. However, even considering combinations of confounders it is unlikely that confounding alone explains the relationship between power frequency EMFs and childhood leukemia.

Because of the rather small relative risks of around two for average exposure to ≥ 3 to 4 mG magnetic flux density or very high current codes there is, however, a possibility that bias due to a combination of confounding and other errors account for the increased risk. It will be shown in the last section that the most important aspect is the exposure metric. A much higher risk may be associated with exposure to power frequency fields. If this is actually the case the problem of bias of other provenience disappears.

Because the increased risk from high levels of exposure to power frequency EMFs is found all over the world a confounder explaining this increased risk must not be quite strong and associated with magnetic fields of various sources but must also be present everywhere in the world. It is virtually impossible that such a risk factor has not yet been detected. Therefore, confounding alone as an explanation for the relationship with leukemia can practically be ruled out.

C. Exposure misclassification

Disregarding chance variations, non-differential exposure misclassification (i.e. misclassification that does not depend on disease status) always leads to an underestimation of the risk. The methods applied to calculate or measure MF in the residences of children are unlikely producing a bias that depends on the disease status (they have usually been done blinded to the case or controls status). Hence, if exposure misclassification was present this will rather have reduced the overall risk estimate. Different effects must be considered whether sensitivity (the probability that a child that was exposed is correctly classified as exposed) or specificity (the probability that a child that was not exposed is correctly classified as not exposed) is affected by the assessment method. The bias depends on six parameters (the exposure prevalence, the true odds ratio, the sensitivity and specificity in cases and controls). A thorough analysis of the effect of different types of exposure misclassification reveals that the vast majority of cases result in a bias towards the zero hypothesis. For low exposure prevalence the impact of a lack of specificity is greater than that of a lack of sensitivity, while for large exposure prevalence the opposite is the case. Considering that high levels of magnetic fields have a low prevalence an increase of specificity (i.e. reducing the number of false positives) has a greater impact on the reduction of bias than of increasing sensitivity (i.e. reducing the number of false negatives). This could explain why odds ratios tend to increase if longer measurements are applied.

Overall, exposure misclassification is a very unlikely cause of a bias in the direction of a higher odds ratio.

D. Selection bias

In studies that were relying on individual measurements selection bias may have played an important role. Participation rates were sometimes lower in controls and especially for families with lower SES. Schüz et al. (2001b) calculated in a simulation study that about two thirds of the increased risk could be due to selection bias. Although Wartenberg (2001) applying a meta-regression could not establish any aspect of study methodology that could account for the variation across studies, it is possible that the proportion of children exposed to high levels of MF has been underestimated in some studies.

The biased odds ratio can be factored into the true odds ratio and a bias factor. The bias factor is often called the selection odds ratio. It can be estimated if there are some data on exposure for non-participants. In the study from Brazil (Wünsch-Filho et al. 2011) measurements of magnetic flux density at the front door of participating and non-participating cases and controls have been conducted that allow computation of the bias factor. It turned out to be 1.08, which indicates a slight bias towards an increased risk. The specific conditions of the study in Brazil (e.g. restriction to cases and controls that did not move to a district outside Sao Paulo, inclusion of children less than 9 years, differences in age distribution of participants and non-participants) do not allow generalization to other studies. However, due to the fact that studies that were registry based obtained essentially the same results speak against a distorting selection bias.

E. Exposure metric

After measurements of MF over 24 hours or even longer periods were introduced lower risk estimates for measured fields as compared to estimates from wire codes were noted. This observation was termed the “wire code paradox”. Although much of the discrepancies disappeared after the pooled analyses (Ahlbom et al., 2000; Greenland et al., 2000), and also the comprehensive meta-analysis of Wartenberg (2001) could find no support for a systematic effect, still in some investigations there was indeed a stronger relationship to estimates from wire codes as compared to measurement. Bowman et al. (1999) and Thomas et al. (1999) published a thorough analysis of this aspect based on data of the Californian childhood leukemia study (London et al., 1991). They correctly noted the different error structure associated with measured fields and calculated fields from the wire codes that are more stable over time. They further pointed to the fact that the bias introduced by basing the risk estimate on exposure variables that are unbiased but prone to statistical variation will be towards the null. It can be shown that this bias is inversely related to the conditional variance of the exposure metric. Hence the higher the variance of the used exposure metric, conditional on the true one, the greater the bias of the risk estimate.

Up to now most considerations put forward were directed towards identification of factors and methodological issues that would explain a spurious relationship between power frequency EMFs and childhood leukemia. Hardly anyone asked the question: "Why is the risk estimated so low?" This question should, however, been asked because there are a number of intriguing facts: First of all, in developing countries with low levels of electrification childhood leukemia incidence is manifold lower as compared to industrialized regions (Parkin et al., 1998). Although registry data in developing countries are less reliable and sparse the difference is too pronounced to be due to underreporting. The time trend of childhood leukemia in industrialized countries suggests that childhood leukemia in the age group below 4 to 5 years of age is essentially a new phenomenon that emerged in the 1920s. Milham and Ossiander (2001) suggest that the acute lymphoblastic leukemia peak is due to electrification. Given the evidence of the pooled analyses, risk increases as a function of average MF flux density reaching significance at the far end of the exposure distribution for children exposed to an average of 3 to 4 mG. This result is clearly not in line with the hypothesis that much if not all of childhood leukemia (at least for the most prevalent ALL type in the age group of 2 to 4 years) is due to power frequency EMFs. Obviously there are two conclusions possible: either the hypothesis is wrong or the data must be reinterpreted.

Another difficulty arises due to the fact that animal studies and in vitro tissue culture investigations provided equivocal evidence for a causal relationship between power frequency EMFs and cancer. There is a fundamental problem in clarifying the etiological role of the exposure in the development of leukemia. According to present theory (Greaves 1999; 2002; 2003; 2006; Wiemels et al., 1999) childhood leukemia is a consequence of several (at least two) genetic events one of which already occurred before birth. Factors affecting childhood leukemia may therefore be related to different critical exposure windows: the preconceptional, the prenatal, and the postnatal period. Preconceptional factors may affect the mother and the grandmother during pregnancy with the mother, as well as the father during spermatogenesis. During the prenatal period exposure of the mother during pregnancy and exposure of the fetus may differentially affect the first stage of the disease. In fact, there is evidence that at birth around 1% of children show genetic deviations in cord blood cells (Wiemels et al., 1999; Eguchi-Ishimae et al., 2001; Mori et al., 2002) that could lead to leukemia conditional on them surviving and on additional genetic or epigenetic events. While the frequency of these deviations at birth might have been overestimated it is still manifold higher than the cumulative probability of childhood leukemia. Given this higher incidence of early genetic events, a causal factor for childhood leukemia need not be directly genotoxic and not even mutagenic. A slight but continuous shift of the balance towards survival and proliferation of deviating clones will be sufficient to dramatically increase the incidence. Experimental investigations were generally insufficient to cover such effects.

Assuming that there is an exposure metric, intimately connected to average magnetic flux densities, and actually related to that condition responsible for the increased incidence of childhood leukemia, how does such a metric look like? Actually it is easy to derive the necessary conditions for such an exposure metric from bias considerations. There are only two such conditions that must be met:

a. The conditional expectancy $E(x|z) = z$ (or equal to a linear function of z); where x is the unknown exposure metric and z is the logarithm of the true average magnetic flux density the child is exposed to.

b. The conditional variance $V_{x|z}$ must be inversely related to z.

Based on the pooled analysis of Ahlbom et al. (2000) and assuming average magnetic flux density follows a log-normal distribution with mean 0.55 mG and a geometric standard deviation of 1, using the complete data set of cases and controls, the results of the pooled analysis can be reconstructed. However, *by varying the magnitude of the variance and the slope of the logistic function relating the purported exposure metric to the probability of developing childhood leukemia up to 80% of all cases can be attributed to the exposure.*

Fig.1 shows one of such Monte Carlo analyses. It can be seen that the bias of the risk estimate related to average MF flux density decreases as the level increases, however, the bias with respect to the assumed exposure metric reaches a factor of about 25 at levels above the third quartile. Of course, the precision of the actual measurements is much lower than indicated in the figure that is constructed by sampling from a theoretical log-normal distribution. However, this does not affect the validity of the argument since imprecisions in the average flux density lead to a bias towards 1. Therefore, the argument even holds in the absence of a relevant imprecision in measurements. The simulation was performed in such a way that exactly the same number of cases and controls are allocated to the average flux density categories as reported in Ahlbom et al. (2000) while varying the relationship between the theoretical alternative exposure metric that has the features a. and b. outlined above. Assuming that this correct metric is causally related to childhood leukemia, attributable fractions between 1% and 80% are calculated dependent on the relationship between the average MF flux density and this assumed metric.

While of course this analysis does not prove the assumption that most of childhood leukemia is due to electrification, it demonstrates that the data obtained so far do not contradict this assumption. It is of crucial importance to analyze existing measurement data for aspects of the exposure that are in line with conditions a. and b. stated above. These exposure conditions may be analyzed by in vitro studies to asses their potential the facilitate transformation of already genetically damaged cells.

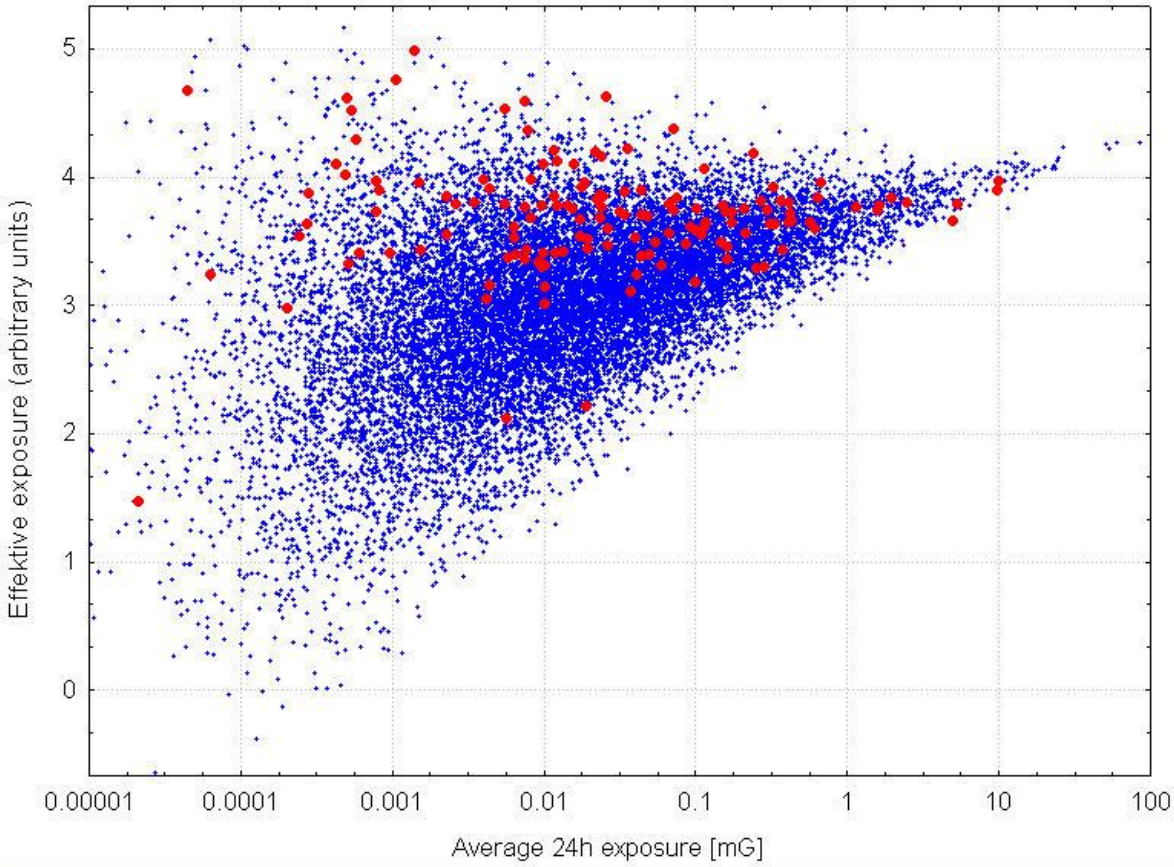

Fig. 1: Results of Monte Carlo simulation under the assumption of a log-normal distribution of average magnetic flux densities in the homes of children that are related to an assumed ‚effective' exposure metric that follows the conditions a. and b. mentioned in the text. Blue are controls and red children with leukemia. The purported ‚effective' exposure metric is associated with an attributable fraction of 80% and the odds-ratio for the highest quartile is around 50.

IV. CONCLUSIONS

The only endpoint studied so far in sufficient detail is childhood leukemia. Brain and nervous system tumors were also studied in some detail but due to the diversity of these tumors no conclusions can be drawn.

Childhood leukemia is the most frequent childhood malignancy that peaks in the age group of 2 to about 5 years. This peak seems to have been newly evolved in the early quarter of the 20th century and may be due to electrification. This assumption is supported by the absence of this peak or it being much less pronounced in developing countries.

An overview of existing evidence from epidemiological studies indicates that there is a continuous increase of risk with increasing levels of average magnetic field exposure. Risk estimates reach statistical significance at levels of 3 to 4 mG. A low number of children are exposed at these or higher levels.

As an alternative interpretation of the association of leukemia with power frequency MF contact currents have been put forward (Kavet et al. 2000). Indeed, considering that a correlation between the magnitude of contact currents in the homes (e.g. in the bathtub) has been found and dosimetry indicates that high levels of internal fields could exist in the bone marrow of children touching metallic water fixtures, the hypothesis has some empirical support. However, a report from an epidemiological investigation in California (Does et al. 2011) could find no indication that contact currents play a decisive role while results for MF flux densities are in line with the previous findings of an increased risk with increasing exposure to power frequency MF in the homes.

I have pointed out (Kundi 2006) that under four conditions (temporal relation, association, environmental equivalence, and population equivalence) epidemiological evidence alone is sufficient to suggest disease causation. This is in line with the hazard assessment of IARC that specifies the default rule for assessing an agent as carcinogenic if there is sufficient evidence from epidemiological studies. Support from animal experiments or mechanistic studies is not necessary in these cases. Evidence from epidemiological studies is considered sufficient if a positive relationship has been observed between the exposure and cancer in studies in which chance, bias and confounding could be ruled out with reasonable confidence.

In the studies of childhood leukemia and residential exposure to power frequency magnetic fields measurements have been conducted after diagnosis. This is a violation of the condition of temporal relation. However, these measurements can be considered an estimate of the exposure during the etiologically relevant period. But still it would result in some exposure misclassification. Because this type of misclassification is non-differential it can only reduce the observed association. Furthermore, support comes from studies with calculated fields that cover the relevant period. Therefore, the epidemiological evidence can be considered to fulfill the criterion.

Due to the small fraction of homes with very high exposure levels single studies have often insufficient power to detect an effect of the assumed magnitude of a doubling of the risk at levels around 3-4 mG. Therefore, meta-analyses and pooled analyses are important to investigate whether the association is due to chance. These analyses show a statistically significant association. There is no indication of a threshold but some investigations found reduced risks at intermediate levels, which might be due to inconsistencies in the sources that account for these exposure levels. There is sufficient evidence of an association that is apparent based on measurements, calculations, wire codes and other proxies for exposure.

Most studies used matching by at least sex and age, some added other potential confounders like region, SES, number of siblings etc. Care has been applied in most investigations to have the same population base for cases and controls. Studies investigating potential confounders did not reveal any factor other than exposure to power frequency MF that could be responsible for the observed association. There is only one cohort study (Verkasalo et al. 1993). This study, although with only 140 childhood cancer cases, is in line with the assumption of an association. An important analysis using the case-specular method supports the assumption of population and environmental equivalence (Ebi et al. 1999). Because the etiology of childhood leukemia is still not clear it is difficult to directly test the features most relevant for assessing the ceteris paribus condition. One investigation (Yang et al. 2008) indicates that power frequency MF may interact with specific genetic conditions. These results can be interpreted in two ways: the risk of leukemia from exposure to MF may be increased only in individuals harboring some specific polymorphism, on the other hand it is possible that exposure increases the genetic instability independently of an already increased instability due to a genetic polymorphism leading to a greater probability of developing the disease. At present there is no evidence to discriminate between these possibilities. If the first interpretation is valid different fractions of children harboring the relevant genetic condition would result in differences in the observed risk and thus some studies could have violated the population equivalence principle. Only in this case, it would be failure to detect an effect and not a spuriously increased risk. Overall, there is no reason to assume that the principles of population and environmental equivalence has been violated in such a way that spuriously increased risks could have resulted.

For all these reasons it can be concluded that there is sufficient evidence from epidemiological studies of an increased risk from exposure to power frequency MF that cannot be attributed to chance, bias or confounding. Therefore, according to the rules of IARC such exposures can be classified as a group 1 carcinogen.

It has to be stressed, however, that according to the rules of IARC the working groups may up- or down-grade the classification upon consideration of the overall evidence. The IARC working group considered the lack of supporting evidence from animal experiments and in vitro studies as sufficient to down-grade the classification to 2B. Although it is not possible to discuss this aspect in this context, there are several problems with this view: first, there is no animal model for ALL, the most frequent childhood leukemia type; second, animal studies are difficult due to the fact that procedures usually applied, i.e. exposure levels just below the acute toxicity level, cannot be followed for MFs due to muscle and nerve excitations accompanying such exposures; third, at levels relevant for human long-term exposure in vitro experiments would have to detect extremely rare cellular events to account for the increased risk observed in epidemiological investigations, which is impossible using methods available to date. Therefore, strong and consistent support from such studies can neither be expected nor demanded. Consequently, lack of support from such evidence cannot be used as an argument to down-grade the classification based in epidemiology.

Considering the possibility that aspects of exposure to power frequency EMFs that have not yet been detected may account for a greater proportion of cases than assumed there are two necessary steps to be taken: Concerted efforts must be undertaken to scrutinize existing data and collect new ones that should reveal whether or not exposure metrics exist that show the necessary conditions for an effective exposure metric; and, second, precautionary measures must be delineated that result in a reduction of all aspects of exposure to power frequency EMFs.

Exposure guidelines of IEEE and ICNIRP are solely derived from immediate effects such as nerve and muscle excitations. These guidelines are indeed sufficient to protect from such acute effects (although indirect effects from contact currents cannot be ruled out). Evidence for long-term chronic effects has been collected in the past decades and has reached a state that it cannot longer be denied that these effects are real. Only under very exceptional and remote conditions of a combination of several unknown confounders, selection bias and differential exposure misclassification the established relationship could be spurious. These combinations must have been present all over the world. There is no other risk factor identified so far for which such unlikely conditions have been put forward to postpone or deny the necessity to take steps towards exposure reduction. As one step in the direction of precaution, measures should be implemented to guarantee that exposure due to transmission and distribution lines is below an average of about 1 mG. This value is arbitrary at present and only supported by the fact that in many studies this level has been chosen as a reference.

- The balance of evidence suggests that childhood leukemia is associated with exposure to power frequency EMFs either during early life or pregnancy
- Considering only average MF flux densities the population attributable risk is low to moderate, however, there is a possibility that other exposure metrics are much stronger related to childhood leukemia and may account for a substantial proportion of cases. The population attributable fraction ranges between 1-4% (Kheifets et al., 2007) 2-4% (Greenland & Kheifets 2006), and 3.3% (Greenland 2001) assuming only exposures above 3 to 4 mG are relevant. However, if not average MF flux density is the metric causally related to childhood leukemia the attributable fraction can be much higher. Calculating a guideline level based on the unit-risk approach leads to a level close to 1 mG.
- Other childhood cancers except leukemia have not been studied in sufficient detail to allow conclusions about the existence and magnitude of the risk
- IEEE guideline levels are designed to protect from short-term immediate effects, long-term effects such as cancer seem to be evoked by levels several orders of magnitudes below current guideline levels
- Precautionary measures are warranted that should reduce all aspects of exposure, because at present we have no clear understanding of the etiologically relevant aspect of the exposure

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Table 11- 4: Synopsis of childhood cancer epidemiologic studies (1979 – 2012)

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Wertheimer & Leeper 1979	Greater Denver area, Colorado/ 1950-1973/ Case-control	wire-codes by inspection (not blinded) of surroundings of residences occupied at birth and time of death	retrospective (1976-1977) assessment	all assessments within 22 days	age (m), sex, urbanization, SES, family pattern, traffic	344 cancer deaths (age<19) from files, matched controls from next entry in birth register or from alphabetical list
Fulton et al. 1980	Rhode Island/1964-1978/Case-control	power lines (<45.72m from residences) assessed and MF calculated as combined weighted average (based on Wertheimer-Leeper measurements)	retrospective (1979) assessment	all assessments within same period	age(m), SES	119 leukemia patients (age<20) from Rhode Island hospital files; 240 control addresses from birth register

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Tomenius 1986	Stockholm county/ 1958-1973/ Case-control	inspection of visible electrical constructions within 150m of dwellings occupied at birth and diagnosis date; spot measurements at the door of the dwellings (blinded to case status)	retrospective (~1981) assessment	all assessments within same period	age(m), sex(m), district(m)	716 tumor cases (660 malignant, 56 benign) from cancer registry (age<19), matched controls from entry into birth register just before or after index case from same church district
Savitz et al. 1988	Five-county Denver area, Colorado/1976-1983/Case-control	wire-code of homes occupied prior to diagnosis (blinded to case status); spot measurements at the front door, in child's and parent's bedrooms and other rooms of frequent occupancy; interviews of mothers (in some cases fathers or adopted mothers)	retrospective (~1985) assessment	all assessments within same period	age±3y (m), sex(m), area(m), SES, traffic density, maternal age, maternal smoking	356 cancer cases (age<15) from cancer registry (71% interviewed, 36% measurements, 90% wire codes); 278 controls (79% resp.rate) from RDD (80% interviewed, 75% measurements, 93% wire codes)

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Coleman et al. 1989	Four boroughs near London/1965-1980/ Case-control	historical exposure by type and distance of electricity supply within 100 m of residences; distance to center of building assessed blinded to case status; calculations according to peak winter load of the power lines	retrospective assessment	all assessments within same period	age(m), sex(m), year of diagnosis(m)	84 leukemia cases (age<18) and 141 cancer controls from cancer registry
Myers et al. 1990	Yorkshire/1970-1979/ Case-control	assessment of overhead power lines within a distance depending on type of power line (100-500m) of home at birth; flux densities calculated from line load data and distance to center of dwelling	retrospective (1981-1989) assessment	all assessments within same period	age(m), sex(m), district(m), house type	374 cancer cases (age<15) from registries; 588 controls from nearest entry in birth register of the same district
London et al.	Los Angeles County,	24-h MF	measurements	all	age±1 or 2 or	232 leukemia cases

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
1991	CA/1980-1987/Case-control	measurements (IREQ/ EMDEX) at location of child's bed; EF, MF and static magnetic field spot measurements; Wertheimer-Leeper wire code (all facilities within 46m; blinded to case status); interviews with parents about use of appliances etc.	1987-1989	assessments within same period	3y(m), sex(m), ethnicity(m), indoor pesticides, hair dryers, black&white TV, fathers occupational exposure to chemicals	(70% part.rate) from LA County Cancer Surveillance Program (age<11); 232 matched controls (90% part.rate) – 65 as friends of cases, others by RDD (5 digits cases, last 2 random)
Verkasalo et al. 1993	Finland/ 1970-1989/ Retrospective Cohort	estimated magnetic flux density from high-voltage power lines in the center of the building	cumulative and max. flux density any time between birth and diagnosis	n.a.	age, sex, calendar period	68300 boys and 66500 girls (age<20) identified having lived any time after birth in a house with a distance < 500m from a 110, 220, or 400 kV power line and an estimated flux density exceeding 0.1mG; 140 cancer cases from follow-up in cancer registry through 1990.
Feychting &	Sweden/1960-	calculations (blinded)	the year	all	age(m), sex(m),	142 cancer cases within

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Ahlbom 1993	1985/Nested Case-control	based on historical load data, wire configuration and distance from 220 and 400kV power lines and spot measurements (several rooms, 5-min measurements, main current turned on and off)	closest to date of diagnosis	assessments within same period	parish(m), year of diagnosis, apartment/single house, traffic (NO_2)	the study base of children (age<16) living on a property <300m from any 220 or 400kV power line; 558 matched controls from the study base.
Olsen et al. 1993	Denmark/1968-1986/ Case-control	calculations based on estimated historical load of overhead transmission lines, transmission cables, and substations (50-400 kV)	retrospective up to 9 mo before birth	all assessments within same period	age(m), sex(m)	1707 cancer cases from registry (age<15) and 4788 matched controls from population register
Fajardo-Gutierrez et al. 1993	Mexico City/not specified/Case-control	interview with parents including assessment of distance and type of transmission and distribution lines, power substations etc.	n.a.	n.a.	age±2y(m), SES	81 leukemia cases from two hospitals; 77 controls from orthopedics or traumatology department

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Coghill et al. 1996	England/1986-1995/ Case-control	E- and H-field probes designed for the study measured 24 h in the bedroom; data used only for the period 20:00 to 08:00	retrospective	parallel measurements in case and control homes	age(m), sex(m)	56 leukemia cases (age<15) from various sources (media advertising, self-help groups, Wessex Health Authority) and 56 controls
Gurney et al. 1996	Seattle area, Washington/1984-1990/Case-control	wire-code by inspection of homes (blinded for case status) occupied within 3 y before diagnosis, electrical appliances by interview with mothers and mailed questionnaire	retrospective (1989-1994) assessment	all assessments within same period	age±2y(m), sex(m), area of residence(m), race, mothers education, family history of brain tumors, ETS, living on a farm, head/neck x-ray, head injury, epilepsy, fits	133 brain-tumor cases (age<20) (74% part.rate) by Cancer Surveillance System; 270 controls by RDD (79% part.rate)

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Preston-Martin et al. 1996	Los Angeles County, California/1984-1991/ Case-control	wire-code and outside spot measurements of homes occupied from conception to diagnosis (blinded for case status); 24h measurements in child's bedroom and another room for a subset; electrical appliances, occupation etc. by interviews with mothers	retrospective (1990-1992) assessment	all assessments within same period	age±1y(m), sex(m), year of diagnosis, SES, parents occupation, building type	298 brain tumor cases (age<20) (68% part.rate); 298 controls by RDD (70% part.rate)
Tynes & Haldorsen 1997	Norway/1965-1989/Nested Case-control	cohort (age <15) living in a ward crossed by a high-voltage power line (≥45kV in urban, ≥100kV in rural areas) in at least one of the years 1960, 1970, 1980, 1985, 1987, 1989.	Calculated historical fields	n.a.	age(m), sex(m), municipality(m), SES, type of building, number of dwellings	500 cancer cases (94%) from cancer registry; 2004 controls (95%) randomly selected from cohort

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Petridou et al. 1997	Greece/1993-1994/Case-control	distance to transmission and distribution lines, field calculation	n.a.	n.a.	age(m), sex(m), region(m), maternal age, education etc.	117 childhood leukemia cases (age<15) (77% of eligible) and 202 controls (68% of eligible)
Michaelis et al. 1997a	Lower Saxony, Germany/1988-1993/ Case-control	24h measurements (EMDEX II) in the child's bedroom and living room in dwellings where the child lived longest (not blinded to case status); perimeter measurements (measurement wheel) with recordings every foot (~30cm) when walking through the rooms and outside the house where the child lived for at least 1 y.	measurements 1992-1995	all measurements within same period	age±1y(m), sex(m), SES, urbanization	129 leukemia cases (age<15) (59% part.rate) from register; 328 controls (167 from same district, 161 from random district) (53% part.rate) from government registration files

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Michaelis et al. 1997b	Berlin/1991-1994/ Case-control (pooled with data from Michaelis et al. 1997a)	as above	not specified	not specified	age±1y(m), sex(m), SES, urbanization, age at diagnosis, West/East Germany	47 leukemia cases (age<15) (59% part.rate) from register; 86 controls (28% part.rate) from government registration files
Linet et al. 1997	Illinois, Indiana, Iowa, Michigan, Minnesota, New Jersey, Ohio, Pennsylvania, and Wisconsin/1989-1994/Case-control	24h measurements (EMDEX C) in child's bedroom (blinded to case status); spot measurements in the residences and at the front door; wire coding of residences of residentially stable case-control pairs	~2 years	all measurements within same period	age(m), ethnicity(m), 8-digits phone number(m), sex, SES, time of measurem., urbanization, type of residence, birth order, birth weight, mother's age, medical x-ray	638 ALL cases (age<15) from register of Children's Cancer Group (78% part.rate); 620 controls from RDD (63% part.rate).

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Li et al. 1998	Taipei Metropol.Area (3 districts), Taiwan/ 1987-1992/ Ecological	high voltage transmission lines (69 -345kV) were mapped to 124 administrative regions; households with ≥50% intersecting a buffer zone of 100m around transmission lines	n.a.	n.a.	age (5y groups), calendar year	28 leukemia cases from registry in a study base of ~121.000 children (age<15); 7 cases within 21 cases outside a 100m corridor each side of a transmission line
Dockerty et al. 1998	New Zealand/1990-1993/Case-control	24h measurements (Positron) in child's bedroom and another room (only for leukemia cases); interview with mothers	1-2 years	all measurements within same period	age(m), sex(m), SES, maternal smoking, living on a farm	303 cancer cases (age<15) from 3 registries (88% part.rate) – 121 leukemia cases; 303 controls from birth register (68% part.rate)

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
UKCCS 1999	England, Scotland & Wales/1991(92)-1994(96)/Case-control	spot measurements (EMDEX II) in child's bedroom, 90 min measurements in main family room, 48h measurements (20% of case-control pairs) at child's bedside; school measurements; weighted averages from info obtained by questionnaire; adjustments from historical load data	~2 years	<4 months in 98% of case-control pairs (spot), within 4 weeks (48h measurem.)	age (m), sex(m), district(m), deprivation index	2226 cancer cases (age<15) from registry (59% part.rate); 2226 matched controls from registry
McBride et al. 1999	Canada (5 provinces)/ 1990-1994(95)/Case-control	48h personal measurements (Positron), 24h measurements in child's bedroom	9 months average	2 months average	age±3-6mo (m), sex(m), area(m), maternal age, maternal education,	399 leukemia cases (age<15) (90% part.rate) from treatment centers and registry; 399 matched

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
		(75% cases, 86% controls); wire codes (78% cases, 85% controls) and residence perimeter and front door measurements (64% cases, 74% controls) (blinded to case status) (EMDEX C); interviews with parents			income, ethnicity, number of residences	controls (76% part.rate) from health insurance/family allowance rolls
Green et al. 1999a	Greater Toronto Area, Canada/1985-1993/ Case-control	48h personal measurements (Positron); spot measurements in child's bedroom and two other rooms; wire codes; interviews with parents	2-3 y average	~5 mo average	age±1y (m), sex(m), family income, siblingship, residential mobility, insecticides, mother's medication and exp. prior or during pregn.	201 leukemia cases (age<15) from hospital record (64% part.rate); 406 controls from telephone marketing list (10,000 residences) (63% part.rate)
Green et al. 1999b	Greater Toronto Area, Canada/1985-1993/ Case-control	as above	2-3 y average	~5 mo average	as above	88 leukemia cases (age<15) from hospital record; 133 controls

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
						from telephone marketing list (10,000 residences)
Schüz et al. 2001a	West Germany/1993(90)-1997(94)/Case-control	24h measurements (FW2a) under mattress of child's bed; 24h measurements (EMDEX II) in living room; perimeter measurements with recordings every foot (~30cm) when walking through the rooms			age(m), sex(m), community(m), SES, year of birth, urbanization, residential mobility, season, type of residence	514 leukemia cases (age<15) from cancer registry (61% of eligible) and 1301 controls from population registry (61% of eligible)
Schüz et al. 2001b	Lower Saxony/1988 – 1993 & Western Germany/1992-1994/ Case-control	as above			age(m), sex(m), community(m), SES, urbanization	64 cases of CNS tumors (age<15) from registry and 414 controls from population registry

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Mizoue et al. 2004	Japan/1992-2001/Ecological	classification of 294 districts according to their proximity to high voltage power lines (66 and 220V); proportion of area of district (0%, <50%, >50%) within ±300m of a power line	n.a.	n.a.	age (5y groups)	14 cases (age<15) of hematopoietic malignancies identified from two hospitals (all that treated these malignancies)
Draper et al. 2005	England & Wales/ 1962-1995/Case-control	computed distance from nearest overhead power line (132kV, 275kV, 400kV) of residence at birth	n.a.	n.a.	age±6mo(m), sex(m), district(m), SES	29081 cancer cases (age<15) identified from several registries (88% of total); 29081 controls from birth registers
Perez et al. 2005	Cuba (Habana)/1996-2000/Case-control	spot measurements inside and outside (Bell 4090), measurement of ionizing radiation	not specified	not specified	age(m), sex(m), school(m)	unknown number of leukemia cases (age<15) and controls

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Kabuto et al. 2006	Tokyo, Nagoya, Kyoto, Osaka and Kitakyushu metropolitan areas (Japan)/1999-2001/Case-control	7 days continuous MF measurement (EMDEX Lite) in child's bedroom; spot measurements in- and outside the house (EMDEX II)	~13 mo	~3 days	age±(≤)1y(m), sex(m), region(m), population size(m), father's and mother's education	321 ALL/AML cases (age<15) from several registries of childhood cancer study groups (49% part.rate); 634 controls from residential registry (29% part.rate)
Mejia-Arangure et al. 2007	Mexico-City/1995-2003/Case-control	spot measurements (EMDEX II) at the front door; wire coding (blinded to case status)	not specified	not specified	age, sex, SES, birth weight, maternal age, traffic, district, family history of cancer	42 ALL/AML cases (age<16) with Down syndrome from 4 (all) treating hospitals; 124 healthy controls with Down syndrome from 2 centers
Feizi & Arabi 2007	Iran (Tabriz)/1998-2004/Case-control	distance and calculated fields	n.a.	n.a.	age(m), sex(m), SES(m), race(m), district(m)	60 AL cases (83% of eligible) (age<15) and 59 hospital controls (79% of eligible)
Lowenthal et al. 2007	Tasmania/1972-1980/Case-control	distance from power line	n.a.	n.a.	age(m), sex(m)	783 adult and 71 childhood cases of MPD or LPD and matched controls

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Yang et al. 2008	Shanghai/2006-2007/Case-only	distance from transformer or power lines	n.a.	n.a.	age, gender, parental education, pesticides, television set etc. in children's room, chemical factory, telecom transmitter <500 m	123 AML cases (age<15) with or without XRCCI Ex9p16A
Abdul-Rahman et al. 2008	Malaysia/2001-2007/Case-control	distance from power lines and substations (GPS)	n.a.	n.a.	not specified	128 AL cases (age<15) and 128 hospital controls
Malagoli et al. 2010	Italy (Modena, Reggio Emilia)/1986-2007/	calculated fields from power lines ≥132 kV	n.a.	n.a.	age(m), sex(m), municipality(m), parent education, income	64 cases (age<14) of hematological malignancies and 256 controls
Kroll et al. 2010	England, Wales/1962-1995/Case-control	calculated fields from overhead power line (132kV, 275kV, 400kV) of residence at birth	n.a.	n.a.	age(m), sex(m), district(m)	28968 cancer cases (age<15)
Sohrabi et al. 2010	Iran (Teheran)/2007-2009/Case-control	distance to power lines (123, 230, 400 kV) using GPS	n.a.	n.a.	age(m), sex(m)	300 ALL cases (age<18) and 300 hospital controls

Study	Country/Period/Study Type	Exposure assessment	Interval diagnosis - measurement	Interval measurement cases-controls	Confounders considered & matching variables(m)	Case/control selection
Saito et al. 2010	Japan/1999-2002/Case-control	1-week measurement (EMDEX Lite) near bedside	Not specified	12.4 days	age(m), sex(m), region(m), population size(m), mother education	55 childhood brain tumor cases (age<15) and 99 controls
Does et al. 2011	California/2004-2007/Case-control	30 min measurement of contact current in the bathtub , indoor spot measurements (EMDEX Lite)	28 months	8 months	age, sex, race, income	245 leukemia cases (95% of eligible) (age<8) and 269 controls (92% of eligible)
Wünsch-Filho et al. 2011	Brazil (Sao Paulo)/2003-2009/Case-control	24 h measurements (EMDEX II) under the child's bed, distance to power lines	Not specified	Not specified	age(m), sex(m), city of birth(m),race, mobility,etc.	179 ALL cases (age<9) (90% of contacted) and 565 controls (88% of contacted)

RDD…Random Digit Dialing, n.a…not applicable, MF…magnetic field, SES…socio-economic status, ALL…acute lymphoblastic leukemia, AML…acute myeloid leukemia, AL…acute leukemia, LPD…lymphoproliferative disorders, MPD…myeloproliferative disorders

Table 11- 5: Synopsis of main results of childhood cancer studies (1979 – 2012)

Study	Endpoint	Exposure category	Outcome [95% CI]
Wertheimer & Leeper 1979[a]	Leukemia	LCC* (birth address)	
		HCC	OR 2.28 [1.34 – 3.91]
	Lymphoma	LCC*	
		HCC	OR 2.48 [0.73 – 8.37]
	Nervous system tumors	LCC*	
		HCC	OR 2.36 [1.03 – 5.41]
	Others	LCC*	
		HCC	OR 2.38 [0.93 – 6.06]
	All hematopoietic	LCC*	
		HCC	OR 2.31 [1.41 – 3.77]
	All cancers	LCC*	
		HCC	OR 2.33 [1.59 – 3.42]
Fulton et al. 1980	Leukemia	Very low*[c]	
		Low	OR 1.1 [0.5 – 2.4]
		High	OR 1.2 [0.6 – 2.6]
		Very high	OR 1.0 [0.5 – 2.3]
Tomenius 1986	Leukemia	no 200 kV-line*	
		200 kV-line<150m	OR 1.09 [0.29 – 4.12]
	Lymphoma	no 200 kV-line*	
		200 kV-line<150m	OR 1.48 [0.35 – 6.35]
	Nervous system tumors	no 200 kV-line*	
		200 kV-line<150m	OR 3.96 [0.85 – 18.52]
	Others	no 200 kV-line*	
		200 kV-line<150m	OR 2.59 [0.70 – 9.66]
	All hematopoietic	no 200 kV-line*	
		200 kV-line<150m	OR 1.26 [0.47 – 3.34]
	All cancers	no 200 kV-line*	
		200 kV-line<150m	OR 2.15 [1.12 – 4.11]

Study	Endpoint	Exposure category	Outcome [95% CI]
	All cancers	<3mG birth dwelling*	
		≥3mG	OR 2.67 [1.18 – 6.08]
	All cancers	<3mG diagn. dwelling*	
		≥3mG	OR 2.60 [1.20 – 5.67]
Savitz et al.1988	Leukemia	<2mG low power use*	
		2+ mG	OR 1.93 [0.67 – 5.56]
	Lymphoma	<2mG low power use*	
		2+ mG	OR 2.17 [0.46 – 10.31]
	Brain tumors	<2mG low power use*	
		2+ mG	OR 1.04 [0.22 – 4.82]
	Others	<2mG low power use*	
		2+ mG	OR 0.96 [0.31 – 2.98]
	All hematopoietic	<2mG low power use*	
		2+ mG	OR 1.99 [0.57 – 5.14]
	All cancers	<2mG low power use*	
		2+ mG	OR 1.35 [0.63 – 2.90]
	Leukemia	<2mG high power use*	
		2+ mG	OR 1.41 [0.57 – 3.50]
	Lymphoma	<2mG high power use*	
		2+ mG	OR 1.81 [0.48 – 6.88]
	Brain tumors	<2mG high power use*	
		2+ mG	OR 0.82 [0.23 – 2.93]
	Others	<2mG high power use*	
		2+ mG	OR 0.75 [0.30 – 1.92]
	All hematopoietic	<2mG high power use*	
		2+ mG	OR 1.51 [0.68 – 3.35]
	All cancers	<2mG high power use*	
		2+ mG	OR 1.04 [0.56 – 1.95]

Study	Endpoint	Exposure category	Outcome [95% CI]
	All cancers	0-0.64 mG low power use*	
		0.65-0.99 mG	OR 1.28 [0.67 – 2.42]
		1.0-2.49 mG	OR 1.25 [0.68 – 2.28]
		2.5+ mG	OR 1.49 [0.62 – 3.60]
	All cancers	0-0.64 mG high power use*	
		0.65-0.99 mG	OR 1.13 [0.61 – 2.11]
		1.0-2.49 mG	OR 0.96 [0.56 – 1.65]
		2.5+ mG	OR 1.17 [0.54 – 2.57]
	Leukemia	LCC*	
		HCC	OR 1.41 [0.57 – 3.50]
	Lymphoma	LCC*	
		HCC	OR 1.81 [0.48 – 6.88]
	Brain tumors	LCC*	
		HCC	OR 0.82 [0.23 – 2.93]
	Others	LCC*	
		HCC	OR 0.75 [0.30 – 1.92]
	All hematopoietic	LCC*	
		HCC	OR 1.51 [0.68 – 3.35]
	All cancers	LCC*	
		HCC	OR 1.04 [0.56 – 1.95]
	All cancers	UG 2y before diagnosis*	
		VLCC	OR 0.96 [0.39 – 2.34]
		OLCC	OR 1.17 [0.65 – 2.08]
		OHCC	OR 1.40 [0.71 – 2.75]
		VHCC	OR 5.22 [1.18 – 23-09]
	All cancers	VLCC/OLCC*^{b}	
		UG	OR 0.89 [0.51 – 1.55]
		OHCC	OR 1.25 [0.67 – 2.31]
		VHCC	OR 4.66 [0.95 – 22.76]

Study	Endpoint	Exposure category	Outcome [95% CI]
Coleman et al. 1989	Leukemia	≥100 m nearest substation*	
		50-99 m	OR 0.75 [0.40 – 1.38]
		25-49 m	OR 1.49 [0.61 – 3.64]
		0-24 m	OR 1.63 [0.32 – 8.38]
Myers et al. 1990	All cancers	<0.1mG*	
		0.1-0.3mG	OR 0.96 [0.37 – 2.51]
		≥0.3mG	OR 1.73 [0.59 – 5.07]
London et al. 1991	Leukemia	<0.68mG* (24h.measurem.)	
		0.68-1.18mG	OR 0.68 [0.39 – 1.17]
		1.19-2.67mG	OR 0.89 [0.46 – 1.71]
		≥2.68mG	OR 1.48 [0.66 – 3.29]
		<0.32mG (spot bedroom)*	
		0.32-0.67mG	OR 1.01 [0.61 – 1.69]
		0.68-1.24mG	OR 1.37 [0.65 – 2.91]
		≥1.25mG	OR 1.22 [0.52 – 2.82]
		UG/VLCC*	
		OLCC	OR 0.95 [0.53 – 1.69]
		OHCC	OR 1.44 [0.81 – 2.56]
		VHCC	OR 2.15 [1.08 – 4.26]
Verkasalo et al. 1993	Leukemia	≥4mG any time	SIR 1.55 [0.32 - 4.54]
	Lymphoma	≥4mG any time	SIR [0.00 - 4.19]
	Nervous system tumors	≥4mG any time	SIR 2.31 [0.75 - 5.40]
	Others	≥4mG any time	SIR 1.24 [0.26 - 3.62]
	All hematopoietic	≥4mG any time	SIR 1.49 [0.74 - 2.66]
	All cancers	≥4mG any time	SIR 1.66 [0.34 - 4.84]
Feychting & Ahlbom 1993	Leukemia	<1mG* (calculated)	
		1-2mG	OR 2.1 [0.6 – 6.1]

Study	Endpoint	Exposure category	Outcome [95% CI]
		≥2mG	OR 2.7 [1.0 – 6.3]
	Lymphoma	<1mG* (calculated)	
		1-2mG	OR 0.9 [0.0 – 5.2]
		≥2mG	OR 1.3 [0.2 – 5.1]
	Nervous system tumors	<1mG* (calculated)	
		1-2mG	OR 1.0 [0.2 – 3.8]
		≥2mG	OR 0.7 [0.1 – 2.7]
	Others	<1mG* (calculated)	
		1-2mG	OR 1.6 [0.6 – 4.3]
		≥2mG	OR 0.2 [0.0 – 1.7]
	All hematopoietic	<1mG* (calculated)	
		1-2mG	OR 1.7 [0.6 – 4.5]
		≥2mG	OR 2.2 [1.0 – 4.7]
	All cancers	<1mG* (calculated)	
		1-2mG	OR 1.5 [0.7 – 2.9]
		≥2mG	OR 1.1 [0.5 – 2.1]
Olsen et al. 1993	Leukemia	<1mG* (calculated)	
		1-4mG	OR 0.3 [0 – 2.0]
		≥4mG	OR 6.0 [0.8 – 44]
	Lymphoma	<1mG* (calculated)	
		1-4mG	OR 5.0 [0.7 – 36]
		≥4mG	OR 5.0 [0.3 – 82]
	CNS tumors	<1mG* (calculated)	
		1-4mG	OR 0.4 [0.1 – 2.8]
		≥4mG	OR 6.0 [0.7 – 44]
	All three combined	<1mG* (calculated)	
		1-4mG	OR 0.7 [0.2 – 2.0]
		≥4mG	OR 5.6 [1.6 – 19]

Study	Endpoint	Exposure category	Outcome [95% CI]
Fajardo-Gutierrez et al. 1993	Leukemia	Transformer station[d]	OR 1.56 [0.73 – 3.30]
		High voltage power line	OR 2.63 [1.26 – 5.36]
		Electric substation	OR 1.67 [0.65 – 4.35]
		Transmission line	OR 2.50 [0.97 – 6.67]
Coghill et al. 1996	Leukemia	< 5 V/m E-field *	
		5-9 V/m	OR 1.49 [0.47 – 5.10]
		10-19 V/m	OR 2.40 [0.79 – 8.09]
		≥20 V/m	OR 4.69 [1.17 – 27.78]
Gurney et al.1996	Brain tumors	UG*	
		VLCC	OR 1.25 [0.74 – 2.13]
		OLCC	OR 0.74 [0.34 – 1.61]
		OHCC	OR 1.07 [0.55 – 2.06]
		VHCC	OR 0.51 [0.16 – 1.60]
		LCC*	
		HCC	OR 0.86 [0.50 – 1.48]
Preston-Martin et al. 1996	Brain tumors	0.09-0.51 mG Md 24h *	
		0.52-1.02 mG	OR 1.5 [0.7 – 3.2]
		1.03-2.03 mG	OR 1.8 [0.7 – 4.5]
		2.04-10.4 mG	OR 1.2 [0.4 – 3.2]
		VLCC/OLCC*	
		UG	OR 1.9 [1.0 – 3.6]
		OHCC	OR 0.8 [0.6 – 1.2]
		VHCC	OR 1.2 [0.6 – 2.1]
Tynes & Haldorsen 1997	Leukemia	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 1.8 [0.7 – 4.2]
		≥1.4mG	OR 0.3 [0.0 – 2.1]
	Lymphoma	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 1.0 [0.1 – 8.7]
		≥1.4mG	OR 2.5 [0.4 – 15.5]

Study	Endpoint	Exposure category	Outcome [95% CI]
	Nervous system tumors	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 1.9 [0.8 – 4.6]
		≥1.4mG	OR 0.7 [0.2 – 2.1]
	Others	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 2.9 [1.0 – 8.4]
		≥1.4mG	OR 1.9 [0.6 – 6.0]
	All hematopoietic	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 1.4 [0.7 – 3.1]
		≥1.4mG	OR 0.7 [0.2 – 2.4]
	All cancers	<0.5mG (TWA birth-diagn)*	
		0.5-1.4mG	OR 1.9 [1.2 – 3.3]
		≥1.4mG	OR 1.0 [0.5 – 1.8]
Petridou et al. 1997	Leukemia	Very Low*	
		Low	OR 0.99 [0.54–1.84]
		Medium	OR 1.84 [0.26–12.81]
		High	OR 4.26 [0.94–19.44]
		Very high	OR 1.56 [0.26–9.39]
Michaelis et al. 1997a	Leukemia	<2mG (Median 24h)*	
		≥2mG	OR 3.2 [0.7 – 14.9]
		<2mG (Median night)*	
		≥2mG	OR 3.9 [0.9 – 16.9]
Michaelis et al. 1997b (pooled with previous)	Leukemia	<2mG (Median 24h)*	
		≥2mG	OR 2.3 [0.8 – 6.7]
		<2mG (Median night)*	
		≥2mG	OR 3.8 [1.2 – 11.9]

Study	Endpoint	Exposure category	Outcome [95% CI]
Linet et al. 1997	ALL	<0.65mG (TWA)*	
		0.65-1mG	OR 0.96 [0.65 – 1.40]
		1-2mG	OR 1.15 [0.79 – 1.65]
		2-3mG	OR 1.31 [0.68 – 2.51]
		3-4mG	OR 1.46 [0.61 – 3.50]
		4-5mG	OR 6.41 [1.30 – 31.7]
		≥5mG	OR 1.01 [0.26 – 3.99]
Li et al.1998	Leukemia	≥100m from transm.line	
		<100m	SIR 2.43 [0.98 – 5.01]
		Total population<15y	
		≥100m from transm.line	SIR 1.05 [0.64 – 1.58]
		<100m	SIR 2.69 [1.08 – 5.55]
Dockerty et al. 1998	Leukemia	<1mG (24h bedroom AM)*	
		1-2mG	OR 1.4 [0.3 – 7.6]
		≥2mG	OR 15.5 [1.1 – 224]
		<1mG (24h daytime room)*	
		1-2mG	OR 3.7 [0.7 – 18.8]
		≥2mG	OR 5.2 [0.9 – 30.8]
UKCCS 1999	Leukemia	<1mG (estim.AM exp.)*	
		1-2mG	OR 0.78 [0.55 – 1.12]
		2-4mG	OR 0.78 [0.40 – 1.52]
		≥4mG	OR 1.68 [0.40 – 7.10]
	Central nervous system cancers	<1mG (estim.AM exp.)*	
		1-2mG	OR 2.44 [1.17 – 5.11]
		2-4mG	OR 0.70 [0.16 – 3.17]
		≥4mG	OR --
	Others	<1mG (estim.AM exp.)*	

Study	Endpoint	Exposure category	Outcome [95% CI]
		1-2mG	OR 0.81 [0.52 – 1.28]
		2-4mG	OR 1.08 [0.45 – 2.56]
		≥4mG	OR 0.71 [0.16 – 3.19]
	All cancers	<1mG (estim.AM exp.)*	
		1-2mG	OR 0.93 [0.72 – 1.19]
		2-4mG	OR 0.87 [0.53 – 1.42]
		≥4mG	OR 0.89 [0.34 – 2.32]
McBride et al. 1999	Leukemia	<0.8mG (lifetime predicted)*	
		0.8-1.5mG	OR 0.74 [0.48 – 1.13]
		1.5-2.7mG	OR 1.15 [0.70 – 1.88]
		≥2.7mG	OR 1.02 [0.56 – 1.86]
		Low (Kaune-Savitz)*	
		Medium	OR 1.12 [0.77 – 1.64]
		High	OR 1.17 [0.74 – 1.86]
Green et al. 1999a	Leukemia	<0.4mG (spot measurem.)*	
		0.4-0.9mG	OR 0.47 [0.12 – 1.89]
		0.9-1.5mG	OR 0.75 [0.19 – 3.02]
		≥1.5mG	OR 1.47 [0.44 – 4.85]
Green et al. 1999b	Leukemia	<0.3mG (48h measurem.)*	
		0.3-0.7mG	OR 2.0 [0.6 – 6.8]
		0.7-1.4mG	OR 4.0 [1.1 – 14.4]
		≥1.4mG	OR 4.5 [1.3 – 15.9]
		<0.4mG (spot measurem.)*	
		0.4-0.8mG	OR 1.8 [0.5 – 6.1]

Study	Endpoint	Exposure category	Outcome [95% CI]
		0.8-1.6mG	OR 2.8 [0.8 – 10.4]
		≥1.6mG	OR 4.0 [1.2 – 13.6]
Schüz et al. 2001a	Leukemia	<1mG (Md 24h)*	
		1-2mG	OR 1.15 [0.73 – 1.81]
		2-4mG	OR 1.16 [0.43 – 3.11]
		≥4mG	OR 5.81 [0.78 – 43.2]
		<1mG (Md night-time)*	
		1-2mG	OR 1.42 [0.90 – 2.23]
		2-4mG	OR 2.53 [0.86 – 7.46]
		≥4mG	OR 5.53 [1.15 – 26.6]
Schüz et al. 2001b	CNS tumors	<2mG (Md 24h)*	
		≥2mG	OR 1.67 [0.32 – 8.84]
		<2mG (Md night-time)*	
		≥2 mG	OR 2.60 [0.45 – 14.9]
Mizoue et al. 2004	All hematopoietic	0% area intersection*	
		<50%	IRR 1.6 [0.5 – 5.1]
		>50%	IRR 2.2 [0.5 – 9.0]
Draper et al.2005	Leukemia	≥600m (from power line)*	
		200-600m	RR 1.22 [1.01 – 1.47]
		<200m	RR 1.68 [1.12 – 2.52]
	Brain tumors	≥600m (from power line)*	
		200-600m	RR 1.18 [0.95 – 1.48]
		<200m	RR 0.74 [0.47 – 1.15]
	Others	≥600m (from power line)*	
		200-600m	RR 0.96 [0.82 – 1.12]
		<200m	RR 0.88 [0.62 – 1.25]
Perez et al. 2005	Leukemia	<1mG*	
		1 mG	OR 1.46

Study	Endpoint	Exposure category	Outcome [95% CI]
		5 mG	OR 6.72
		10 mG	OR 45.15
Kabuto et al. 2006	ALL+AML	<1mG (1wk TWA)*	
		1-2mG	OR 0.93 [0.51 – 1.71]
		2-4mG	OR 1.08 [0.51 – 2.31]
		≥4mG	OR 2.77 [0.80 – 9.57]
	ALL+AML	<1mG (1wk night-time)*	
		1-2mG	OR 0.97 [0.52 – 1.79]
		2-4mG	OR 1.08 [0.47 – 2.47]
		≥4mG	OR 2.87 [0.84 – 9.88]
	ALL	<1mG (1wk TWA)*	
		1-2mG	OR 0.87 [0.45 – 1.69]
		2-4mG	OR 1.03 [0.43 – 2.50]
		≥4mG	OR 4.67 [1.15 – 19.0]
Mejia-Arangure et al. 2007	ALL+AML	<1mG (spot)*	
		1-4mG	OR 0.94 [0.37 – 2.4]
		4-6mG	OR 0.88 [0.15 – 5.1]
		≥6mG	OR 3.7 [1.05 – 13]
		Low (Kaune-Savitz)*	
		Medium	OR 5.8 [0.92 – 37]
		High	OR 4.1 [0.66 – 25]
Feizi & Arabi 2007	Leukemia	≤4.5mG*	
		>4.5mG	OR 3.60 [1.11 – 12.39]
Lowenthal et al. 2007	LPD+MPD	>300 m from power line*	
		0-300 m (at age 0-15)	OR 3.23 [1.26 – 8.29]
Yang et al. 2008	AL with XRCC1 Ex9 + 16A allele	>500 m from power line*	

Study	Endpoint	Exposure category	Outcome [95% CI]
		0-500 m	OR 2.37 [0.94–5.97]
		>100 m from power line*	
		0-100 m	OR 4.31 [1.54–12.08]
		>50 m from power line*	
		0-50 m	OR 4.39 [1.42–13.54]
Abdul-Rahman et al. 2008	Leukemia	>200 m from power line*	
		0-200 m	OR 2.30 [1.18–4.49]
Malagoli et al. 2010	All hematological malignancies	<1mG*	
		≥1mG	OR 2.4 [0.4-15.0]
	Leukemia	<1mG*	
		≥1mG	OR 6.7 [0.6-78.3]
	ALL	<1mG*	
		≥1mG	OR 5.3 [0.7-43.5]
Kroll et al. 2010	Leukemia	<1mG*	
		1-2mG	OR 2.00 [0.50–7.99]
		2-4mG	0 case/ 2 controls
		≥4mG	OR 2.00 [0.18–22.04]
	CNS/brain tumors	<1mG*	
		1-2mG	OR 0.50 [0.09–2.73]
		2-4mG	1 case/ 0 control
		≥4mG	OR 0.33 [0.03–3.20]
	Other cancers	<1mG*	
		1-2mG	OR 0.33 [0.07–1.65]
		2-4mG	OR 1.00 [0.14–7.10]
		≥4mG	OR 5.00 [0.58–42.80]
Sohrabi et al. 2010	ALL	>400 m from power line*	
		0-400 m	OR 2.75 [1.59 – 4.76]
Saito et al. 2010	Brain tumors	<1mG bedroom*	
		1-2mG	OR 0.74 [0.17–3.18]
		2-4mG	OR 1.58 [0.25–9.83]

Study	Endpoint	Exposure category	Outcome [95% CI]
		≥4mG	OR 10.9 [1.05–113]
Does et al. 2011	Leukemia	<0.25mV contact current	
		0.25-1.5mV	OR 0.98 [0.63 – 1.53]
		≥1.5mV	OR 0.99 [0.65 – 1.52]
		<0.1mG*	
		0.1-0.2mG	OR 0.96 [0.57 – 1.62]
		0.2-0.5mG	OR 1.23 [0.74 – 2.04]
		≥0.5mG	OR 1.18 [0.71 – 1.96]
Wünsch-Filho et al. 2011	ALL	≥600 m from power line*	
		200-600 m	OR 0.69 [0.28–1.71]
		100-200 m	OR 1.67 [0.49–5.75]
		<100 m	OR 1.54 [0.26–9.12]
		≥600 m from power line*	
		200-600 m (never moved)	OR 0.91 [0.25–3.25]
		100-200 m	OR 3.68 [0.68–19.82]
		<100 m	OR 1.52 [0.11–21.24]

[*] Reference category
[a] Computed from table 5 of the original publication (could be biased due to not considering individual matching)
[b] Computed from table 5 of the original publication
[c] Quartiles of exposure distribution of controls (exposure calculated)
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OR…odds-ratio, SIR…standardized incidence ratio, RR…relative risk, IRR…incidence rate ratio, LCC…low-current code, HCC…high-current code, UG…underground cable, VLCC…very low current code, OLCC…ordinary low current code, OHCC…ordinary high current code, VHCC…very high current code, Md…median, TWA…time weighted average, AM…arithmetic mean, ALL…acute lymphoblastic leukemia, AML…acute myeloid leukemia, LPD…lymphoproliferative disorders, MPD…myeloproliferative disorders

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 13

ELF MF – Melatonin Production – Alzheimer's Disease and Breast Cancer

2012 Updated Chapter

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Prepared for the BioInitiative Working Group

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SECTION 1: UPDATE INTRODUCTION

It has been over 5 years since the publication of the initial BioInitiative in 2007. During that time the BioInitiative web site has been accessed by a considerable number of individuals worldwide: ***(Provide viewing figures.)*** Unfortunately, "pro-industry" representatives from industry itself, from government, and from academia have continued their campaign, despite all evidence to the contrary, against any possible serious ill effects of exposure to extremely low frequency (ELF) magnetic fields (MF) at levels experienced in occupational and residential settings. These pro-industry representatives simply argue that the evidence is insufficient because some epidemiologic studies are negative and some are positive and that there are no biologically confirmed causal pathways. As we showed in the earlier 2007 original BioInitiative publications, the negative studies have serious flaws while the positive studies do not have such flaws. In addition, we discussed two biological pathways related to Alzheimer's disease and breast cancer, which have plausibility based on scientific studies. A third suggested pathway is discussed in this update.

In this chapter update, we provide the following:

1. descriptions and evaluations of newly published epidemiologic studies relating occupational ELF MF exposure to the risk of (a) Alzheimer's disease (AD) and/or dementia, (b) breast cancer;
2. updates related to the three proposed or suggested pathways from ELF MF exposure to AD or dementia:
    a. increased peripheral and brain production of amyloid beta;
    b. decreased production of melatonin; and
    c. ELF MFs may cause chromosome instability, resulting in chromosome segregation errors and increased mutational loads;
3. a discussion of the potential increase in cellular production of amyloid beta (associated with the risk of AD) due to low melatonin production;
4. an update of the relationship between low melatonin production and the risk of breast cancer;

STRUCTURE OF THE UPDATED REPORT

New material is incorporated into the body of the Report. New and revised text and table additions are presented with a red text color.

EXECUTIVE SUMMARY

Melatonin Production

Melatonin is a hormone produced primarily by the pineal gland, located in the center of the brain. Melatonin is evolutionarily conserved and is found in nearly all organisms. It has numerous properties which indicate that it helps prevent both Alzheimer's disease and breast cancer. There is strong evidence from epidemiologic studies that high (≥ 10 milligauss or mG)* that long-term exposure to extremely low frequency (ELF, ≤ 60 Hz) magnetic fields (MF) is associated with a decrease in melatonin production(Section II.)

Alzheimer's Disease

Amyloid beta (Aβ) protein is generally considered the primary neurotoxic agent causally associated with Alzheimer's disease (AD). Aβ is produced by both brain and peripheral cells and can pass through the blood brain barrier.

1. There is longitudinal epidemiologic evidence that high peripheral blood levels of Aβ, particularly $A\beta_{1\text{-}42}$, is a risk factor for Alzheimer's disease (AD). (Section III.A.)
2. There is epidemiologic evidence that extremely low frequency (ELF, 50-60 Hz) magnetic field (MF) exposure up-regulates peripheral blood levels of Aβ. (Section III.A.)
3. There is evidence that melatonin can inhibit the development of AD and, thus, low melatonin may increase the risk of AD (Section III.B.)
4. There is strong epidemiologic evidence that significant (i.e., high), occupational ELF MF exposure can lead to the down-regulation of melatonin production. The precise components of the magnetic fields causing this down-regulation are unknown. Other factors which may influence the relationship between ELF MF exposure and melatonin production are unknown, but certain medications may play a role. (Section II.)
5. There is strong epidemiologic evidence that high occupational ELF MF exposure is a risk factor for AD, based on case-control studies which used expert diagnoses and a restrictive classification of ELF MF exposure. (Section III.C.)
6. There are no epidemiologic studies of AD and radiofrequency MF exposure, only one epidemiology study of non-acute radiofrequency MF exposure and melatonin. There are studies of "AD mice" and radiofrequency exposure (Sections III.D and II.) So, no conclusions concerning health consequences due to exposure are currently possible.

Breast Cancer

The only biological hypothesis which has been epidemiologically investigated to explain the relationship between ELF MF exposure and breast cancer is that high* ELF MF exposure can lower melatonin production, which in turn can lead to changes in the various biological systems which melatonin influences, including increased estrogen production and subsequent deleterious interactions with DNA, decreased antiproliferative activities, increased oxidative DNA damage, and immune response capabilities. Thus lowered melatonin production can be expected to lead to increased risk of breast cancer.

1. *In vitro* and animal studies have demonstrated that (i) melatonin is a potent scavenger of oxygen and nitrogen radicals that cause DNA damage, (ii) melatonin interferes with

estrogen's deleterious interactions with DNA, and (iii) melatonin inhibits the development of mammary tumors. (Section IV.A.)

2. A study published in 2009 (Davanipour et al.) evaluated guanine DNA/RNA damage in relation to melatonin production among 55 mother-father-adult daughter triples who were relatively healthy for their age. The lower melatonin production among the mothers was associated with higher guanine DNA damage. Lower melatonin production among the fathers was marginally associated with guanine damage in either DNA or RNA.
3. Human studies indicate that ELF MF exposure can decrease melatonin production. (Section II.)
3. Human studies have found that low melatonin production is a likely risk factor for breast cancer. (Section IV.B.)
4. Human studies have shown that light-at-night and night shift work reduce melatonin production and are both risk factors for breast cancer. (Section IV.D.)
5. Occupational studies indicate that high ELF MF exposure increases the risk of breast cancer. This is particularly true for a recent, large, and well-designed study from Poland (funded by the NCI, administered for the NCI by Westat, and conducted by Polish scientists).
6. A recent, large, and well-designed, Swedish case-control study used a new ELF MF job exposure matrix, developed by the same group, which is nearly completely at odds with earlier exposure classifications. The female occupation generally thought to be the one with the highest ELF MF exposure (seamstress) was considered to have medium-low exposure, while several lower ELF MF exposed occupations were considered high. The case-control study consequently found no risk associated with high ELF MF occupations as rated by the new matrix, but did find that seamstresses had a statistically elevated risk of breast cancer. This job exposure matrix is likely inappropriate in many important instances and needs to be thoroughly reviewed. (Section IV.E.)
7. Studies of residential ELF MF exposure and breast cancer have been generally negative. Measured residential ELF MF exposure may not be related to actual individual exposure. Residential exposure is most often low, is usually not measured in residences that may be related to the latency period of breast cancer, does not take into consideration point sources of strong magnetic fields which may be related to real exposure, and thus often does not relate to actual exposure. Residential exposure studies are therefore not considered to be of importance for the purposes of this report. (Section IV.F.)
8. Quality radiofrequency studies are lacking. (Section IV.G.)

Seamstresses

As a group, seamstresses have proven to constitute an important occupation for the demonstration of a relationship between ELF MF exposure and both Alzheimer's disease and breast cancer. Seamstresses who use industrial sewing machines have very high and relatively constant ELF MF exposure, particularly those seamstresses working in the apparel industry. This is because the motors of older AC machines are large and produce high levels of ELF MFs, and are on and producing such fields even when no sewing is being done. The AC/DC transformers of DC industrial machines always produce a high field even when the machine is turned off (but not unplugged). In addition, rooms, in which a large number of such machines are used, even have relatively high ambient ELF MF levels. Home sewing machines generally produce smaller ELF MFs, but even these weaker ELF MFs are substantial.

RECOMMENDATION Using the Precautionary Principal, mitigating exposure is a proper goal. Mean occupational exposures over 10 mG or intermittent exposures above 100 mG should be lowered to the extent possible. In situations where this is not feasible, the daily length of exposure should curtailed. Lowering ELF MF exposure can be done by improved placement of the source(s) of magnetic fields (e.g., electric motors in sewing machines, AC/DC converters), shielding, and redesign. It is clear that re-engineering products can greatly lessen ELF MF exposure, and possibly result in important innovations. It is noted that certain automotive models produce medium to high ELF MFs, as do steel-belted radial tires (Milham *et al.*, 1999).

I. INTRODUCTION

All of the studies discussed have based exposure classifications using magnetic field (MF) measurements, not electric field (EF) measurements. We separately discuss extremely low frequency (ELF, ≤ 60 Hz) MFs and radiofrequency (RF) MFs. Furthermore, the discussion is primarily limited to investigations related to ELF MF exposure as a possible risk factor for Alzheimer's disease (AD), female breast cancer (BC), and the possible biological pathways linking ELF MF exposure to AD and BC incidence, e.g., reduction in the production of melatonin.

Exposure Concerns

Epidemiologic investigations are sensitive to errors in exposure assessment and errors in case-control designation. This is particularly true for ELF MF exposure and for AD classification. With respect to occupational exposures, all job exposure matrices (JEM) are based on the measurement of a relatively small number of subjects in each job type. However, extensive measurements have been performed for workers in the electric utility industry and for seamstresses. Note, however, that the Swedish breast cancer study by Forssén *et al.* (2005) used only 5 essentially part-time seamstresses to determine exposure classification (Forssén *et al.* (2004).

The geometric mean ELF MF exposure over the time period of observation is generally used for classification. For ordinal classifications, individual subjects in jobs with mean ELF MF exposure measured close to a boundary value, e.g., between low and medium or between medium and high ELF MF exposure, will frequently be incorrectly classified. This misclassification will generally lead to bias in the estimated risk towards 1, i.e., no risk.

For residential exposures, which do not include living near high power lines, measurements of necessity need to be taken at the current residence. Measurements are usually taken in several rooms at various locations, sometimes with and without electrical equipment turned on, but rarely (if ever) with water lines turned on. Thus, individualized exposures, e.g., sitting near a fuse box, being near one or more AC/DC transformers, use of specific brands and models of home sewing machines, being near a microwave oven in operation, and a myriad of other point sources are missed. Previous residences are usually not available for measurements. Consequently, exposure classification is problematic for studies interested in risk associated with residential ELF MF exposure.

* Unless otherwise specified, "high" ELF MF exposure as used in this report means an exposure of at least 10 mG or (relatively frequent) intermittent exposure above 100 mG,

while "medium" exposure is an average exposure of between 2 and 10 mG or (relatively frequent) intermittent exposure above 10 mG. "Long-term exposure" means exposure over a period of years. Often, other researchers use a cut-point of around 2-3 mG, or sometimes even less, as a "high" average. The reviews of each study presented here detail the specific cut-point(s) used.

** Also, unless otherwise specified, "high" ELF MF exposure as used in this report means an exposure of at least 10 mG, while exposure means exposure over a period of years. **

Diagnostic Concerns

AD is difficult to correctly diagnose. Non-specialists frequently incorrectly diagnose a patient as having AD. Exposure assessment and case-control classification errors bias the odds ratio (OR) estimator, when based on dichotomous exposure classification, towards the null hypothesis. When based on three (3) or more classification groups, exposure assessment and case-control classification errors in the types of analyses used most likely also lead to bias towards the null hypothesis.

> With respect to AD, unless the diagnosis is made by experts, there is a very large false positive rate. That is, community-based physicians often incorrectly diagnose dementia (versus depression, for example) and are particularly poor at determining the correct differential diagnosis of dementia. Most subjects with a diagnosis of dementia are simply assumed to have AD. This means that around 40% of all AD diagnoses by physicians who are not experts are incorrect. Diagnostic information on death certificates is even worse. Such a large error in caseness clearly biases the OR estimator towards the null hypothesis. (Many cases of AD go undiagnosed, especially early stage AD. However, this likely does not lead to a significant error rate in classification of controls.)
>
> With respect to breast cancer, the sub-type of breast cancer is generally recorded, e.g., estrogen receptor positive (ER+) or negative (ER-), which may very well be important with respect to ELF MF exposure. However, sub-group analyses have not usually been performed.

Therefore, in reviewing published studies, particular emphasis is placed on these errors or caveats. Studies which assessed occupational exposures and those which assessed residential exposures are both discussed. Various algorithms for "ELF MF exposure" have been used, and these will also be discussed. Not all studies, exposure data, and exposure algorithms are of equal value.

For both AD and BC, a possible biological pathway of particular importance is down-regulation of melatonin production as a result of long-term ELF MF exposure. This is discussed in detail in this review.

A second possible biological pathway relates specifically to Alzheimer's disease. Long-term ELF MF exposure may increase the production of amyloid beta (Aβ), both in the brain and peripherally. Aβ, particularly the form with 42 amino acids (Aβ1-42), is considered the primary neurotoxic compound causing AD. This pathway was proposed by Sobel and Davanipour (1996a). Recent epidemiologic studies have provided some degree of confirmation. A third pathway has been proposed: genomic instability. Thus, ELF MF exposure may be a risk factor for AD through possibly three complementary biological pathways. (See Sections III.A. and III.B.)

There may certainly be other potential biological pathways that will be identified. For example, melatonin interacts with certain cytokines which appear to affect immune responses. This may be relevant to the early elimination of cells which are either pre-malignant or malignant, thus preventing the development of overt breast or other cancers. However, the two primary pathways outlined above can most easily be evaluated in human studies, both population-based studies and clinical trials.

There are also several epidemiologic studies of melatonin production among workers with long-term occupational exposure to magnetic fields and a single study of women with high (vs low) residential ELF MF exposure. These studies generally indicate that long-term ELF MF exposure can lead to lowered melatonin production.

II. ELF Magnetic Field EXPOSURE and MELATONIN ACTIVITY AND PRODUCTION

A. Melatonin Production

> *Conclusion: Eleven (11) of the 13 published epidemiologic residential and occupational studies are considered to provide (positive) evidence that high ELF MF exposure can result in decreased melatonin production. The two negative studies had important deficiencies that may certainly have biased the results. There is sufficient evidence to conclude that long-term relatively high ELF MF exposure can result in a decrease in melatonin production. It has not been determined to what extent personal characteristics, e.g., medications, interact with ELF MF exposure in decreasing melatonin production.*

Eightly-five percent (85%) to 90% of pineal melatonin production is at night. Laboratory-based studies, using pure sinusoidal magnetic fields under experimental conditions have not found an effect on melatonin production (Graham *et al.*, 1996, 1997; *Brainard et al.*, 1999). However, several studies among subjects chronically exposed in occupational and residential environments have found an effect, while a few have not. The lack of an effect in laboratory settings may be because the ELF MF exposure was too "clean" or because the duration of exposure was not sufficiently long, e.g., days, weeks, months.

The evidence indicates that high and ELF MF exposures may lead to a decrease in melatonin production. Whether this decrease is reversible with a cessation of exposure is unknown. The extent of the decrease is hard to evaluate. It is also not yet possible to identify individual susceptibility to such a decrease in melatonin production.

Melatonin production is generally measured using its primary urinary metabolite, 6-sulphatoxymelatonin (aMT6s). Total overnight melatonin production is best estimated using complete overnight urine samples. Creatinine-adjusted aMT6s is slightly more correlated with cumulative melatonin estimates obtained from sequential overnight blood samples than is unadjusted aMT6s (Cook *et al.*, 2000; Graham *et al.*, 1998).

The human studies in occupational or residential environments which identified an effect are summarized below.

Positive Studies

- Assessment in the Finnish Garment Industry As a follow-up component to a Finnish study of ELF MF exposures among garment factory workers, a small study of nighttime melatonin production was carried out (Juutilainen *et al.*, 1999). aMT6s excretion and creatinine were measured using complete overnight urine samples. Seamstresses (n=31), other garment workers (n=8), and non-exposed outside workers (n=21) participated. Observations were taken using complete overnight urine collections beginning on a Thursday night through the first morning void on Friday and on the subsequent Sunday night through the first morning void on Monday. There was very little variation between the two time period observations within each group, indicating that if there is an effect of ELF MF exposure, it does not disappear over the weekend, at least among seamstresses using older industrial alternating current machines. The average Thursday-Friday non-adjusted aMT6s excretion level and the average aMT6s excretion level adjusted for creatinine were both statistically significantly lower ($p< 0.05$) among the workers in the garment factory compared to the controls, even after controlling for other factors associated with a lowering of melatonin levels: creatinine-adjusted aMT6s - 16.4 vs 27.4 ng/mg; unadjusted aMT6s - 5.1 vs 10.0 ng. There was no indication of a dose-response relationship among the garment factory workers.

  In a follow-up study, Juutilainen and Kumlin analyzed the same data in conjunction with a dichotomization of a measure of light-at-night (LAN), obtained from items in the original study questionnaire concerning use of a bedroom light at night, street lights outside the bedroom windows, and use of curtains which do or do not let light filter through. There was a significant interaction between the dichotomized ELF MF exposure (high/low, i.e., cases vs controls) and LAN (yes/no). aMT6s was significantly lower for subjects with high ELF MF with or without LAN. In addition, aMT6s was significantly lower among subjects with high ELF MF and LAN exposure versus subjects with high ELF MF and no LAN exposure. Alternatively, aMT6s was essentially identical for subjects with low ELF MF exposure, regardless of the LAN status.

- Washington State Residential ELF MF Exposure and Melatonin Study Women, aged 20 to 74, were selected for a study of the relationship of bedroom 60 Hz magnetic field levels and melatonin production (Kaune *et al.*, 1997a,b; Davis *et al.*, 2001a). Approximately 200 women were recruited based on magnetic field exposure information from a case-control study of breast cancer (PI: S Davis). About 100 women were sought whose bedrooms were at the high end of magnetic field level in the original study and about 100 were sought who were at the low end. Concurrent measurements of light at night in the bedrooms of these women were also obtained using a specially modified EMDEX II system. Mean magnetic field levels in the two groups differed by less than 1 mG. Thus, compared to ELF MF exposures in many occupations, the women had quite low ELF MF exposures. However, there was an inverse association between bedroom magnetic field levels and urinary aMT6s adjusted for creatinine levels on the same night, after adjusting for time of year, age, alcohol consumption, and use of medications. The association was strongest at those times of the year with the longest length of daylight and in women who were using medications that themselves were expected to attenuate melatonin production, e.g., beta and calcium channel blockers and psychotropic drugs.

- Crossover Trial of ELF MF Exposure at Night and Melatonin Production Davis *et al.* (2006) conducted a randomized crossover trial among 115 pre-menopausal women with regular periods between 25 and 35 days apart, a body mass index between 18 and 30 kg/m^2, not using hormonal contraceptives or other hormones for at least 30 days before the study period, no history of breast cancer, no history of chemotherapy or tamoxifen therapy, not having been pregnant or breast-feeding within the previous year, not working any night shifts, not taking supplemental melatonin, phytoestrogens or isoflavones, and not eating more than 5 servings of soy-based foods within any one week. ELF MF exposure or sham exposure was for 5 consecutive days. A random half of these women received ELF MF exposure and then sham exposure one month later. The other random half had the exposures reversed. Ovulation was determined in the first, second and third months. The initial exposure (ELF MF or sham) was in the second month during days 3-7 post-ovulation. The second exposure (sham or ELF MF) was during the same days in the third month. The charging base of an electric toothbrush which produced a steady magnetic field was used. It was placed under the subject's bed at the head level so that the subject's head received 5-10 mG exposure above baseline. Complete overnight urine samples were collected on the night of the last exposure (ELF MF or sham) in each of the two exposure periods. There were 2 subjects who did not ovulate during either exposure month and 13 who did not ovulate in one of the two months. Statistical adjustment was made for age, hours of darkness, body mass index, medication use, any alcohol consumption, and number of alcoholic beverages consumed. Because each subject was her own control, these adjustments probably did not affect the point estimates much. A regression analysis was undertaken. The 95% confidence interval (CI) of the regression slope was [-3.0 – +0.7] for all subjects and [-4.1 – -0.2] when the 15 subjects with "minor" protocol violations were eliminated from the analysis. These violations were (a) more than 40 days between the two assessments, (b) urine collections not on the same post-ovulation day, and (c) menstrual period started early. Only (b) appears to be really relevant because these subjects could have had less ELF MF exposure. However, this information is not provided. Separate analyses were conducted for "medication users" (n=14) and non-users (n=101). The slope point estimate for the users was numerically smaller (-3.1) than for the non- users (-1.0). The authors state that the study "found that nocturnal exposure to 60-Hz magnetic fields 5 to 10 mG greater than ambient levels in the bedroom is associated with decreased urinary concentrations of (aMT6s)". It should be noted that the p-value of the slope estimate in the primary analysis (all participants) was greater than 0.05. However, the 95% CI, [-3.0 – +0.7], was quite unbalanced, with 0 being much closer to the upper end of the CI than the lower end. Also, the 95% CI, when the 15 subjects with minor protocol violations are eliminated is entirely below 0, and thus the point estimate is statistically significant at the 0.05 level. The authors also state the following: "(t)he more pronounced effect of magnetic field exposure on melatonin levels seen in medication users and in those with an anovulatory cycle suggest {sic} that individuals who have decreased melatonin levels already may be more susceptible to the effects of magnetic field exposure in further decreasing melatonin levels." The justification for this statement is not based on statistical testing.

- Residential High Power Lines, ELF MF Exposure and aMT6s in the Quebec City Study Levallois *et al.* (2001) evaluated aMT6s among 221 women living near 735-kV power lines compared to 195 age matched women who live far away from such lines. The subjects wore magnetic field dosimeters for 36 consecutive hours to measure their actual ELF MF exposure. The geometric mean 24-hour ELF MF exposure was 3.3 mG among women living near a high power line and 1.3 mG among those who did not live near a high power line. Similarly, geometric mean exposure during sleep was 2.9 mG versus 0.8 mG for the two groups. No direct effect of ELF MF exposure on creatinine-adjusted aMT6s was identified. However, living near a high power line and ELF MF exposure interacted with age and body mass index (BMI; kg/m^2). Living near a high power line was associated with a significant decline in creatinine-adjusted aMT6s among older subjects and subjects with higher BMI. There were similar significant decreases related to age and BMI for women in the lowest quartile versus highest quartile. All analyses were adjusted for age, BMI, alcohol consumption in the previous 24 hours, medication use in the previous 24 hours, light at night, and education.

- Assessment in the Electric Utility Industry Burch *et al.* (1996, 1998, 1999, 2000, 2002) have reported on the association between levels of occupational daytime magnetic field exposure, non-work ELF MF exposure, and the excretion of total overnight and daytime aMT6s among electric utility workers in several studies. These studies are among the largest to evaluate the relationships between ELF MF exposure and melatonin production in humans, and are the only studies to use personal exposure monitoring of both ELF MF and ambient light with a repeated measures design.

    - ✓ In their 1996 abstract, analyses were conducted for 35 of 142 electric utility workers enrolled in a larger study. ELF MF exposure was assessed continuously at 15 second intervals for three 24-hour periods, with logs kept to identify work, sleep and other non-work time periods. Ambient light intensity was also individually measured. Complete overnight urine samples and post-work spot urine samples were collected at the same times over the 3 days. There were statistically significant inverse relationships between nocturnal aMT6s levels and log- transformed worktime mean ELF MF exposure (p=0.013), geometric work-time mean ELF MF exposure (p=0.024), and cumulative work-time ELF MF exposure (p=0.008). There was no association, however, between sleep time and other time ELF MF exposure levels and aMT6s levels during the daytime or nighttime, even though average cumulative ELF MF levels were only somewhat higher during work: 18.3 mG-hours (work); 13.1 mG-hours (non-work); 12.6 mG-hours (sleep).

    - ✓ In their 1998 study, further results related to nocturnal aMT6s urinary excretion in relation to ELF MF exposure were presented, using all 142 electric utility workers. The ELF MF exposure metrics were geometric mean intensity, a rate-of-change metric (RCM), and the standardized rate-of-change metric (RCMS). RC was used as a measure of intermittence, while RCMS was used as a measure of the temporal stability of the serially recorded personal ELF MF exposures. Statistical adjustments were made for age, month, and personal ambient light exposure. 24-hour mean ELF MF exposure intensity, RCM, and RCMS were not associated with either nocturnal aMT6s or creatinine-adjusted aMT6s. However, there was an inverse relationship between residential RCMS and nocturnal aMT6s. The interaction between residential intensity and RCMS was inversely associated with total overnight urinary aMT6s excretion and with creatinine-adjusted nocturnal aMT6s excretion. There was a "modest" reduction in nocturnal aMT6s with more temporally stable ELF MF exposures at work. The effect on nocturnal aMT6s was greatest when residential and workplace RCMS exposures were combined. The authors concluded that their study provides evidence that temporally stable ELF MF exposure (i.e., lower RCMS) are associated with decreased nocturnal urinary aMT6s levels. Given the strong correlation between cumulative overnight serum melatonin levels and both total overnight urinary aMT6s and creatinine-adjusted aMT6s levels, these results indicate a reduction in overnight melatonin production.

✓ In their 1999 study, data from the same 142 electric utility workers were further analyzed. Personal exposure to workplace geometric mean and RCMS were evaluated for their effect on post-work urinary aMT6s measurements. No association between creatinine-adjusted aMT6s and the geometric mean ELF MF exposure, before or after adjustment for age, calendar month and light exposure was found. However, ELF MF temporal stability was associated with a statistically significant reduction in adjusted mean post-work aMT6s concentrations on the second (p=0.02) and third (p=0.03) days of observation. Light exposure modified the ELF MF exposure effect. Overall, there was a significant (p=0.02) interaction between RCMS and ambient light exposure. Reductions in post-work aMT6s levels were associated with temporally stable ELF MF exposures among workers in the lowest quartile of ambient light exposure (mostly office workers), whereas there was no RCMS effect among workers with intermediate or elevated ambient light exposure.

✓ In their 2000 study, Burch *et al.* examined aMT6s levels among a completely different population of 149 electrical workers, 60 in substations, 50 in 3-phase environments, and 39 in other jobs, using the same data collection strategy as was used in the previous study, but with the added characterization of specific work environments. The rationale for this study was based on previous observations in experimental animals suggesting that non-linear field polarization was critical in the reduction of melatonin production. These types of fields were expected to be present within substations and in the vicinity of 3-phase electrical conductors. Other conductors (1-phase, linear polarization) were selected as a control condition because they had not previously been associated with an alteration of melatonin production in laboratory animal studies. Thus, participating workers recorded the times they spent in these environments over the 3-day data collection period. Comparisons were made separately for subjects working in substation or 3-phase environments, or among those working in 1-phase environments. Adjusted mean aMT6s levels were compared statistically among workers in the lowest and highest tertiles of ELF MF exposure, using either the geometric mean or the RCMS measurements. Among workers in either a substation or 3-phase environment for more than 2 hours, nocturnal aMT6s decreased 43% (p=0.03) when tertiles were based on geometric mean exposure and decreased 42% (p=0.01) when tertiles were based on RCMS. With RCMS tertiles, total overnight aMT6s excretion also decreased 42% (p=0.03) and post-work creatinine-adjusted aMT6s decreased 49% (p=0.02). With geometric mean tertiles, total overnight aMT6s excretion decreased 39% and post-work creatinine-adjusted aMT6s decrease 34%. However, neither of these decreases was statistically significant. No ELF MF-related effects were observed among workers with less than 2 hours time spent in substation/3-phase environments. Similarly, no reduction in aMT6s levels were observed among workers in 1-phase environments.

- ✓ In 2002, Burch *et al.* studied two consecutive cohorts of electric utility workers using the same data collection strategy to evaluate the effects of cellular telephone use and personal 60 Hz ELF MF exposure on aMT6s excretion. The sample sizes were 149 for Cohort 1 (from the 2000 study) and 77 for Cohort 2. Total overnight and post-work urine samples and self-reported workplace cell phone use were obtained over three (3) consecutive workdays. ELF MF and ambient light exposure were also measured with specially adapted personal dosimeters. The outcome of interest was melatonin production as measured by aMT6s. The cut- point for high versus low cell phone use was 25 minutes per day. Only 5 worker- days of cell phone use more then 25 minutes were reported in Cohort 1 versus 13 worker-days in Cohort 2. No differences in aMT6s production were found in Cohort 1. However, for Cohort 2 there were significant linear trends of decreasing overnight aMT6s and creatinine-adjusted aMT6s levels with increasing cell phone use. There was also a marginally significant increasing trend in post-work creatinine-adjusted aMT6s with increasing cell phone use. Finally, there was a combined effect of cell phone use and ELF MF exposure on aMT6s excretion: among workers in the highest tertile of ELF MF exposure, those who used a cell phone for more than 10 minutes had the lowest overnight aMT6s and creatinine-adjusted aMT6s levels compared to those with lower ELF MF exposure or cell phone use. All analyses used a repeated measures method and were adjusted for age, month of participation, and light exposure.

- Swiss Railway Worker Study Pfluger and Minder (1996) studied 66 railway engineers operating 16.7 Hz electric powered locomotives and 42 "controls". Mean ELF MF exposure at the thorax for the engineers was above 150 mG and approximately 10 mG for the controls. Thus most controls also had high ELF MF exposure, certainly compared to residential and most occupational ELF MF exposures. Morning and early evening (post-work) urine samples were used to measure aMT6s. Evening aMT6s values were significantly lower following work periods (early, normal or late shifts) compared to leisure periods for the engineers, but not for the controls. Also, morning samples did not differ between leisure and work mornings. This indicates that there was at least somewhat of a recovery from the work-time ELF MF exposures. Evening aMT6s values did not differ between work time and leisure time for either engineers or controls. However, there was a rebound in morning aMT6s between a work period and leisure period. Pfluger and Minder did not report the results of a comparison of nighttime aMT6s levels between engineers and controls.

- Video Display Unit Studies Non-panel video display screens, e.g., computer monitors, produce significant ELF MF exposure despite improvements over the last decade or so. Arnetz and Berg (1996) studied 47 Swedish office workers who used video display units (VDU) in their work in the 1980s. Circulating melatonin levels significantly decreased during work, but not during a day of "leisure" in the same environment.

Nighttime melatonin production was not observed. In 2003, Santini *et al.* conducted a similar, but quite small, study of 13 young female office workers, 6 of whom worked for at least 4 hours per day in front of a video screen. Overnight urine samples were used to measure aMT6s. The aMT6s values of the exposed workers was 54% lower (p<0.01) compared to the non- exposed workers.

Negative Studies

- Italian Study of Workers Gobba *et al.* (2006) recruited 59 workers, 55.9% of whom were women, for a study of melatonin production and ELF MF exposure. Actually more workers were recruited, but urine samples for only those subjects who did not get up to urinate during sleep time were assayed. Creatinine-adjusted aMT6s was measured using a Friday morning urine sample and the following Monday morning urine sample. Mean age was 44.4 years (standard deviation, 9.2). Exposure during worktime was measured over a three-day period. The logarithm of the time weighted average (TWA) and the percent of time above 2 mG were used as the measures of exposure. 2 mG was the cut-point between low and high exposure. 52.5% were in the low exposed group; a larger percentage of men than women were in the low exposed group. Occupations included clothing production (n=26), utility companies (14), teachers (6), engineering industry (5), and miscellaneous (8). There were no significant differences in creatinine-adjusted aMT6s values based on the logarithm of the TWA or percent of observations above 2 mG.

- Occupational ELF MF Exposures among 30 Males Subjects in France Touitou *et al.* (2003) studied 15 men exposed to occupational magnetic fields for between 1 and 20 years and age-matched15 controls. All subjects were free of acute or chronic diseases, had regular sleep habits, did not do night work, took no transmeridian airplane flights during the preceding 2 months, took no drugs, were nonsmokers, and used alcohol and coffee in moderate amounts. Furthermore, they did not use electric razors or hair dryers during the study or in the 24 hours prior to blood sampling. All of the 15 ELF MF exposed men worked in high voltage electrical substations. They also lived near substations. None of the controls had an occupation associated with ELF MF exposure. Exposed subjects had a mean exposure of 6.4 mG during work and 8.2 mG during other times. For the control subjects, the mean exposure was 0.04 mG, both during the day and at other times. Blood samples were taken hourly from 8:00 pm until 8:00 am in a standard manner. All urine between these times was collected. Melatonin concentration (pg/ml) was measured in each blood sample. The study was done in the autumn. The 12 hour melatonin blood concentration curves for the exposed and non-exposed subjects are almost identical. The creatinine-adjusted aMT6s levels are also nearly identical. No analyses were conducted based on length of time in the occupation.

B. **Melatonin Activity and ELF MF**

Conclusion: New research indicates that ELF MF exposure, in vitro, can significantly decrease melatonin activity through effects on MT1, an important melatonin receptor.

Girgert et al. (2010) studied the effects of 12 mG 50 Hz ELF MF exposure on signal transduction of MT1 in parental MCF-7 cells and MCF-7 cells transfected with the MT1 gene. MT1 is a high-affinity melatonin receptor and is responsible for many of melatonin's activities. 12 mG is an exposure experienced by individuals in many occupations, e.g., seamstresses and welders. Melatonin, as discussed in this chapter, has many important properties related to cancer prevention and growth, particularly breast cancer, and to the delay or prevention of AD. For proliferation tests, the MT1-negative and MT1-transfected cells were placed in a medium with and without an estradiol solution – estradiol concentrations ranged from 10^{-12} to 10^{-10} moles. $4x10^{-9}$ moles of melatonin were used in a parallel series of estradiol concentrations to evaluate the effect of melatonin. Cell proliferation assays demonstrated that (i) melatonin inhibited cell growth and (ii) 12 mG ELF MF exposure nearly eliminated the effect of melatonin on cell growth. Furthermore, melatonin's growth inhibitory effect was more prominent in the MCF cells transfected with the MT1 receptor than in the cells which were not transfected.

> Girgert et al. (2010) note that several studies designed to evaluate the effects of melatonin in breast cancer cells were negative. They measured the ELF MF produced by various cell incubators and found several that generated approximately 12 mG. They suggest that negative findings may be due to the use of incubators which produce these relatively high fields.

III. ALZHEIMER'S DISEASE

A. Possible Biologic Pathways from ELF MF Exposure to Alzheimer's Disease

A.1. Over-Production of Peripheral Amyloid Beta Caused by ELF MF Exposure

> *Conclusion: There is now evidence that (i) high levels of peripheral amyloid beta are a risk factor for AD and (ii) medium to high ELF MF exposure can increase peripheral amyloid beta. High brain levels of amyloid beta are also a risk factor for AD and medium to high ELF MF exposure to brain cells likely also increases these cells' production of amyloid beta.*

Sobel and Davanipour (1996a) have published a biologically plausible hypothesis relating ELF MF exposure to AD, based on the unrelated work of many researchers in several different fields. The hypothesized process involves increased peripheral or brain production of amyloid beta (Aβ) as a result of ELF MF exposure, and subsequent transportation of peripheral Aβ across the blood brain barrier. Figure 1 provides a schematic outline of the hypothesis. Each step in the proposed pathway is supported by *in vitro* studies.

Two versions of the amyloid beta protein have been identified. They are identical, except one is longer, 42 versus 40 amino acids. These are specified, respectively, by Aβ1-42 and Aβ1-40. Aβ1-42 is considered the more neurotoxic of the two.

This hypothesis has not yet been fully tested. However, two recent studies of elderly subjects and electrical workers, respectively, have provided important initial support. The Mayeux *et al.* (1999, 2003) papers demonstrate that higher levels peripheral Aβ1-42 are a risk factor for AD. The Noonal et al. (2002a) paper demonstrates that ELF MF exposure can increase the peripheral levels of Aβ1-42 and that contemporaneous blood levels of melatonin are inversely associated with peripheral levels of Aβ1-42.

- Mayeux *et al.* (1999, 2003, 2011) conducted a population-based, longitudinal study of elderly subjects who were cognitively normal at baseline and found that higher peripheral blood levels of $A\beta_{1-42}$ were prognostic of subsequent development of AD. The 2003 paper had a longer follow-up period and 282 additional subjects (169 vs 451).

In the first paper, 105 subjects, cognitively normal at baseline, were followed for an average of 3.6 years. The mean age at baseline was 74.3 +/- 5.3 years. Sixty-four (64) subjects developed AD. Table 1 provides the baseline and follow-up means for age, education, $A\beta_{1-42}$, $A\beta_{1-40}$, and the ratio $A\beta_{1-42}/A\beta_{1-40}$. The subjects who developed AD were older at baseline, had nearly two years less education, and higher $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}/A\beta_{1-40}$. All mean differences were significant at the p=0.001 level, except for the ratio, which was significant at the p=0.05 level.

For $A\beta_{1-42}$, the OR for AD, based on the actual $A\beta_{1-42}$ values, was 1.0114, p = 0.006. Thus, for example, the OR for an individual with an $A\beta_{1-42}$ value 10 pg/ml above the cutpoint for the 1st quartile (24.6 pg/ml) is estimated to be $(1.0114)^{10} = 1.12$, an increase of 12%; for an individual with an $A\beta_{1-42}$ value 40 points above this cutpoint, the estimated increase in risk is 57%. A similar analysis for $A\beta_{1-40}$ did not yield a significant result.

Subjects were then divided into quartiles based on their $A\beta_{1-42}$ values. For $A\beta_{1-42}$ there was a highly significant (p=0.004) trend across quartiles. The adjusted odds ratios (OR) for the 2nd – 4th quartiles were 2.9, 3.6, and 4.0, using logistic regression. The latter two were statistically significant at the 0.05 level. The ranges for the 3rd and 4th quartiles were 45.9 – 85.0 pg/ml and > 85.0 pg/ml, respectively. For the 2nd quartile, the significance level of the OR was not provided; however, the 95% confidence interval (CI) was [0.9 – 6.8]. Perhaps because the per unit analysis was not significant for $A\beta_{1-40}$, an analysis using quartiles was not reported.

In the second paper (Mayeux *et al.*, 2003), follow-up of patients was up to 10 years and there were 451 patients who were cognitively normal at baseline, versus 169 in the initial paper. Table 2 contains the same information for this study as is provided in Table 1 for the initial study. Eighty-six (86) of the 451 subjects developed AD. Presumably, the additional subjects had had their peripheral amyloid beta assayed after the submission of the original paper. Again, the $A\beta_{1-42}$ values were divided into quartiles, based on the 451 subjects who were cognitively normal at their last follow-up. The adjusted relative risk (RR) estimates for the 2nd – 4th quartiles were 1.3, 1.9, and 2.4, using Cox survival analysis. The latter two were statistically significant at the 0.05 and 0.006 levels, respectively. The ranges for the 3rd and 4th quartiles were 60.2 – 84.15 pg/ml and ≥ 84.15 pg/ml, respectively. For the 2nd quartile, the significance level of the OR was again not provided; however, the 95% confidence interval (CI) was [0.6 – 2.1].

> The mean levels of $A\beta_{1-40}$, $A\beta_{1-42}$, and $A\beta_{1-42}/A\beta_{1-40}$ at baseline in the second paper were 133.9 pg/ml, 62.2 pg/ml, and 0.50. In the initial paper, the comparable figures were 120.5 pg/ml, 63.2 pg/ml, and 0.57. The means for $A\beta_{1-42}$ and $A\beta_{1-42}/A\beta_{1-40}$ are quite similar in the two studies. However, the means for $A\beta_{1-40}$ are quite different, so there were most likely several subjects who were not in the initial report, and who had $A\beta_{1-40}$ assays which were very high. These subjects were evidently almost all in the cognitively normal group. This is because in the AD groups, the $A\beta_{1-40}$ means were 134.7 and 136.2 pg/ml. However, in the cognitively normal group, the means were

> 111.8 and 133.3 pg/ml. Thus, the additional 260 subjects with did not develop AD (365-105=260) had an average A$\beta_{1\text{-}40}$ of 142.0 pg/ml. Such a large difference is left unexplained in the Mayeux *et al*. (2003) paper.

Mayeux *et al*. (1999) comment that "cerebral deposition of A$\beta_{1\text{-}42}$ is unlikely to result directly from increased plasma A$\beta_{1\text{-}42}$". However, studies by Zlokovic and colleagues provide a basis for concluding that, in fact, peripheral A$\beta_{1\text{-}42}$ is likely to cross the blood brain barrier, perhaps chaperoned by apolipoprotein E (ApoE), particularly the ε4 isoform (see Sobel & Davanipour, 1996a). Currently, the relative amounts of peripheral and cerebral A$\beta_{1\text{-}42}$ or A$\beta_{1\text{-}40}$ which aggregate are unknown.

> Two newly developed PET scan techniques, however, provide the ability to investigate the relative amounts in humans (Klunk *et al*., 2004; Ziolko *et al*., 2006; Small *et al*., 2006). It is also straightforward to use labeled amyloid beta to determine the rate at which peripheral amyloid beta is transported to the brain, at least in animal models and perhaps also in humans.

In 2011, Mayeux and Schupf further discussed their and other researchers findings and their hypothesis that a high blood level of A$\beta_{1\text{-}42}$ is a risk factor for late onset AD, but the A$\beta_{1\text{-}42}$ blood levels decline with advancing dementia. Similarly, blood levels of A$\beta_{1\text{-}40}$ may also decline with disease progression.

- Schupf et al. (2008) studied a sample of 1021 non-demented subjects at least 65 years old at baseline. Plasma A$\beta_{1\text{-}42}$ and A$\beta_{1\text{-}40}$ levels were assayed at baseline. One hundred and four (104; 10.2%) subjects developed AD within 4.6 years. Higher plasma A$\beta_{1\text{-}42}$ at baseline was associated with a 3-fold increase in the risk of AD. On the other hand, development of AD was associated with a significant decline in plasma A$\beta_{1\text{-}42}$ and a decrease in the A$\beta_{1\text{-}42}$/A$\beta_{1\text{-}40}$ ratio as dementia progressed.
- Cosentino et al. (2010) studied a sample of 880 subjects, 65 or older and dementia free at the first of two plasma Aβ measurements. High baseline plasma for both A$\beta_{1\text{-}42}$ and A$\beta_{1\text{-}40}$, and decreasing or stable A$\beta_{1\text{-}42}$ were associated with faster decline in multiple cognitive areas.
- Schupf et al. (2010) studied the relationship between plasma A$\beta_{1\text{-}42}$ and A$\beta_{1\text{-}40}$ levels and the occurrence of dementia among a community-based cohort of 225 Down syndrome adults, dementia-free at baseline. Sixty-one (61, 27.1%) developed AD during follow-up. The mean length of follow-up was 4.1 years. The increase in plasma A$\beta_{1\text{-}40}$, decrease in plasma A$\beta_{1\text{-}42}$, and decrease in A$\beta_{1\text{-}42}$/A$\beta_{1\text{-}40}$ levels were significantly associated with development of dementia. This study was an extension of the follow-up time of an earlier study (Schupf et al., 2007).
- Devanand et al. (2011) studied a small number of patients (n=20) with amnestic mild cognitive impairment (MCI), a harbinger of AD development in the majority of cases, and 19 cognitively normal controls. Plasma A$\beta_{1\text{-}42}$ and A$\beta_{1\text{-}40}$ levels were assayed. In addition PET scans determined Pittsburgh compound B (PiB) binding in various brain locations and in the total brain. The plasma A$\beta_{1\text{-}42}$/A$\beta_{1\text{-}40}$ ratio was decreased in the MCI patients compared to the controls, but A$\beta_{1\text{-}42}$ and A$\beta_{1\text{-}40}$ did not differ between the two groups. PiB binding levels were significantly higher in the cingulate and parietal brain areas and in the entire brain among the MCI patients compared to the

controls. However, in the prefrontal cortex and parahippocampal gyrus the differences were only marginally significant, but the sample size was relatively small. Low $A\beta_{1-42}/A\beta_{1-42}$ and $A\beta_{1-40}$ were associated with high cingulate, parietal and total brain PiB binding, using regression analyses which included age, gender, and cognitive test scores.

- For completeness, we provide the results of a meta-analysis by Song et al. (2011) of 12 cross-sectional and 7 longitudinal studies of plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ levels related to AD. The results were as follows:
  - ✓ Longitudinal studies: cognitively normal subjects who developed AD had higher baseline plasma $A\beta_{1-42}$ and $A\beta_{1-40}$ (p=0.0001 and 0.006, respectively), but non-significantly increased $A\beta_{1-42}/A\beta_{1-40}$ (p=0.10).
  - ✓ Cross-sectional studies: AD patients had marginally significant (p=0.08) lower plasma $A\beta_{1-42}$. The $A\beta_{1-40}$ levels were not significantly different (p=0.69).

- Noonan *et al.* (2002a) examined 60 electric utility workers in studying the relationship between measured ELF MF exposure during the work day and serum $A\beta_{1-42}$ and $A\beta_{1-40}$ (square root transformed) levels. ELF MF exposure was individually determined by wearing a dosimeter at the waist during work time. Blood samples were obtained between 2:50 pm and 4:50 pm. The primary findings were as follows:
  - **i.** there was an inverse association between physical work and A Aβ levels;
  - **ii.** there was an apparent trend for the $A\beta_{1-42}$, $A\beta_{1-40}$, and $A\beta_{1-42}/A\beta_{1-40}$ levels to be higher for higher magnetic field exposure (significance not provided); and
  - **iii.** the differences (Table 3) in Aβ levels between the highest ($\geq$ 2 milliGauss (mG), n=7) and lowest (< 0.5 mG, n=20) exposure categories were 156 vs 125 pg/ml (p=0.10) for $A\beta_{1-40}$, 262 vs 136 pg/m (p=0.14) for $A\beta_{1-42}$, and 1.46 vs 1.03 for $A\beta_{1-42}/A\beta_{1-40}$ (significance not provided).

  There was a 93% increase in $A\beta_{1-42}$, a 25% increase in $A\beta_{1-40}$, and a 42% increase in the ratio $A\beta_{1-42}/A\beta_{1-40}$ between the lowest and highest ELF MF exposure categories. The 2 mG cutpoint for the highest category is the cutpoint generally used for medium (or at times high) ELF MF exposure in epidemiologic studies. Thus, while the sample size was small, this study provides some evidence that ELF MF exposure may result in higher peripheral production of Aβ for exposures above 2mG.

  Melatonin production was estimated using urinary 6-sulphatoxymelatonin (aMT6s) adjusted for creatinine (Graham *et al.*, 1998). aMT6s is the primary urinary metabolite of melatonin. A complete overnight urine sample was used to estimate overnight melatonin production, normally about 85-90% of total 24-hour production. A post-work urine sample, taken on the same day as the post-work blood sample, was used to estimate work time melatonin blood levels. The overnight creatinine-adjusted aMT6s levels were, on average, about 5 times higher than the post-work creatinine-adjusted aMT6s levels. Noonan *et al.* state that the correlations between overnight creatinine-adjusted aMT6s and amyloid beta levels were not significant. No data were provided. However, post-work creatinine-adjusted aMT6s levels were negatively correlated with both the $A\beta_{1-42}$ and the $A\beta_{1-42}/A\beta_{1-40}$ post-work levels. The Spearman correlation coefficients were -0.22 (p=0.08) and -0.21 (p=0.10), respectively. With adjustment for age and physical work, the correlation with $A\beta_{1-42}$ was marginally stronger (-0.25, p=0.057). The timing of the urine sample with respect to the blood sample appears to be important. Table 4 provides the Spearman correlations, adjusted for age and physical work, based on the time difference between blood and urine samples, which were all obtained after the blood draw. Some of the workers had their urine sample in the early evening. It is clear that the correlation is strongest when the samples are taken close to one another in time.

In an unadjusted analysis, the post-work creatinine-adjusted aMT6s levels were split into tertiles. Subjects in the highest tertile had the lowest levels of Aβ1-42, Aβ1-40, and $A\beta_{1\text{-}42}$/ $A\beta_{1\text{-}40}$ (Table 5). However, subjects in the middle tertile had higher levels than subjects in the lowest tertile.

- In an *in vitro* study, Del Giudice *et al.* (2007) used human neuroglioma cells (H4/APPswe), which stably overexpress a specific human mutant amyloid precursor protein (APP, to examine the effect of ELF MF exposure. ELF MF or sham exposure was 3.1 mT (31,000 mG) for 18 hours. Total Aβ and total Aβ1-42 production was statistically significantly elevated among the ELF MF exposed cells compared to the cells with sham exposure. No gross morphological changes or changes in viability were observed in the ELF MF exposed cells. The 3.1 mT exposure level is 2-3 orders of magnitude higher than the highest occupational mean exposures. The authors state that such high levels were administered because occupational exposures are "much more prolonged than the one described in our experimental setting". There was no indication that any longer duration exposure at lower levels was studied.

A.2. Lowered Melatonin Production: An Alternative/Complementary Pathway

Conclusion: There is considerable in vitro and animal evidence that melatonin protects against AD. Therefore it is certainly possible that low levels of melatonin production are associated with an increase in the risk of AD.

Several *in vitro* and animal studies indicate that melatonin may be protective against AD and thus low or lowered melatonin production may be a risk factor for AD. These studies have generally found that supplemental melatonin has the following effects:

- the neurotoxicity and cytotoxicity of A□ is inhibited, including mitochondria (Pappolla *et al.*, 1997, 1999, 2002; Shen YX *et al.*, 2002a; Zatta *et al.*, 2003; Jang *et al.*, 2005);
- the formation of β-pleated sheet structures and Aβ fibrils is inhibited (Pappolla *et al.*, 1998; Poeggeler *et al.*, 2001; Skribanek *et al.*, 2001; Matsubara *et al.*, 2003; Feng *et al.*, 2004; Cheng and van Breemen, 2005);
- the profibrillogenic activity of apolipoprotein E ε4, an isoform conferring increased risk of AD, is reversed (Poeggeler *et al.*, 2001);
- oxidative stress *in vitro* and in transgenic mouse models of AD is inhibited if given early (Clapp-Lilly *et al.*, 2001a; Matsubara *et al.*, 2003; Feng *et al.*, 2006), but not necessarily if given to old mice (Quinn *et al.*, 2005);
- survival time is increased in mouse models of AD (Matsubara *et al.*, 2003);
- oxidative stress and proinflammatory cytokines induced by A□1-40 in rat brain are reduced *in vitro* and *in vivo* (Clapp-Lilly *et al*, 2001b; Shen YX *et al.*, 2002b; Rosales-Corral *et al.*, 2003);
- the prevalence of Aβ1-40 and Aβ1-42 in the brain is decreased in young and middle aged mice (Lahiri *et al.*, 2004);

- memory and learning is improved in rat models of AD pathology (Shen YX *et al.*, 2001; Weinstock and Shoham, 2004), but not necessarily in Aβ-infused rat models (Tang *et al.*, 2002).

Note that transgenic mouse models of AD mimic senile plaque accumulation, neuronal loss, and memory impairment. See Pappolla *et al.* (2000), Cardinali *et al.* (2005), Srinivasan *et al.* (2006), Cheng *et al.* (2006), and Wang and Wang (2006) for reviews. Thus, chronic low levels of melatonin production may be etiologically related to AD incidence.

A.3. Cytogenetic Hypothesis Relating ELF MF Exposure to Alzheimer's Disease

Conclusion: This is an interesting hypothesis and is deserving of research efforts.

Maes and Verschaeve (2011) review evidence that genomic instability, including aneuploidy, telomere shortening, and gene amplification, is associated with an increased risk of early-onset familial AD and perhaps sporadic AD. The authors then discuss possible genetic effects of ELF MF (or electromagnetic field (EMF)) exposure. Further, directed research into this hypothesis is warranted.

D. Epidemiologic Studies of Alzheimer's Disease/Dementia and ELF MF Exposure

Conclusion: There is strong epidemiologic evidence that exposure to ELF MF is a risk factor for AD. There are now twelve (12) studies of ELF MF exposure and AD or dementia which . Nine (9) of these studies are considered positive and three (3) are considered negative. The three negative studies have serious deficiencies in ELF MF exposure classification that results in subjects with rather low exposure being considered as having significant exposure. There are insufficient studies to formulate an opinion as to whether radiofrequency MF exposure is a risk or protective factor for AD.

D.1. Introduction

First, it is necessary to point out that there are no case-control studies of melatonin as a risk factor for AD. This is primarily because AD results in a precipitous decline in melatonin production due to the destruction of specific neuronal structures and therefore it is inappropriate to use "current" melatonin production of cases as a surrogate estimate of the pre-AD melatonin production. Also there have yet to be any longitudinal studies of melatonin production. This is probably because neither urine nor blood have been collected appropriately to measure nocturnal melatonin production.

If ELF MF exposure is a true risk factor, there are several problematic areas in evaluation and comparison of epidemiologic studies related to occupational ELF MF exposure and Alzheimer's disease, particularly the following.

1. Diagnosis – false positive diagnoses will bias the odds ratio estimator towards 1.0
2. Occupational exposure assessment – inclusion of subjects with low exposure in the "exposed" categories likely biases the odds ratio estimator towards 1.0
    - Definition of ELF MF exposure – published studies have differing definitions

of ELF MF exposure, potentially resulting in "exposure" categories with significant proportions of subjects with low exposure

- Cut-points for non-exposure/exposure categories – some studies use numerical estimates of exposure developed from earlier exposure studies (job exposure matrices) in certain occupations and use average estimates and/or low cut-points to determine "medium" exposure
- Ever versus never exposed – at least one study used ever exposed, with a low threshold for exposure
- Categorized occupational data – categorized data from governmental databases leads to relatively large variation in "exposure" within occupational categories, which results in subjects with low exposure being classified as having been exposed.

Table 6 provides the data on the percentages of ELF MF exposed subjects in the published studies to date. There is a wide range of percentages, due primarily to variation in exposure definition, use of average or mean job-specific estimates, and secondarily to the use of varying job exposure matrices. Table 7 provides the odds ratio estimates of studies discussed in some detail below. The studies which used death certificates or other non-expert databases for the identification of AD cases are not included in Table 7.

The role of seamstresses among workers with high occupational ELF MF exposure in the two *et al.* studies (1995, 1996b) and the Davanipour *et al.* study (2007) is discussed.

D.2. Death Certificates-Governmental Databases: Alzheimer's Disease Diagnosis

The use of death certificates or governmental databases to identify AD cases is certainly problematic. False positive diagnoses tend to bias the OR estimator towards 1.0. Most diagnoses of AD have been and still are made by physicians who are not experts in AD, and who seldom have sufficient clinical time to make a proper diagnosis. The determination of dementia and subsequent differential diagnosis of AD by someone other than an expert has a high false positive rate. In addition, many physicians do not think that AD is a "cause of death", which results in an increase in the false negative rate.

Therefore the recent "positive" Feychting *et al.* (2003), Håkansson *et al.* (2003), and Park *et al.* (2005) studies and the "negative" Savitz *et al.* (1998a,b) and Noonan *et al.* (2002b) studies have been excluded from the discussion below of individual studies. The Johansen *et al.* study (2000) has also been excluded because it depended upon the clinical hospital discharge diagnoses of an historical cohort to determine a "diagnosis" of "presenile" AD or "dementia". Evidently, in that study, late-onset (age at least 65) AD was included under "dementia". (It should be noted that Johansen *et al.* found an increased risk of "dementia", but not "presenile" AD, associated with higher ELF MF exposure.)

D.3. ELF MF Exposure Assessment Rates and Analytic Results

The Sobel *et al.* (1995, 1996b), the Davanipour *et al.* (2007), and the Harmanci *et al.* (2003) studies have followed nearly the same protocol for ELF MF exposure assessment and classification into low, medium and high ELF MF occupations. In these studies, medium exposure was defined as mean ELF MF occupational exposure above 2 mG, but less than 10 mG, or intermittent exposures above 10 mG, while high exposure was defined as mean ELF M F exposure above 10 mG or intermittent exposures above 100 mG. The rates of medium or high (M/H) exposure in these studies are considerably lower than the rates in the Feychting *et al.* (1998a), Graves *et al.* ((1999), Qiu *et al.* (2004), and Savitz *et al.* (1998b) studies and somewhat lower than the Feychting *et al.* (2003) study. The remaining three studies (Häkansson *et al.*, 2003; Savitz *et al.*, 1998a; Johansen, 2000) utilized subjects from electrical industries and therefore understandably have high rates of ELF MF exposure. (See Table 6 for these rates.)

Thus, it is likely that a substantial percentage of ELF MF "exposed" subjects in 4 of the 6 comparable studies (Feychting *et al.*, 1998a; Graves *et al.*, 1999; Qiu *et al.*, 2004) (Table 7) had a high rate of somewhat minimal exposure in the "exposed" category, due to classification methodologies, compared to the "exposed" categories in the Davanipour *et al.* (2007), Harmanci *et al.* (2003), and the Sobel *et al.* (1995, 1996b) studies. This would tend to lead to an OR estimate closer to 1.0 in the 4 former studies.

D.3.1. Sobel *et al.* (1995) Study – Positive Study

The initial publication of an apparent association between AD and having worked in occupations with likely ELF MF exposure consisted of three case-control studies, two from Helsinki, Finland, and one from Los Angeles, USA (Sobel *et al.*, 1995). Control groups varied: the first case-control study analyzed used VaD patients; the second (and largest study) used non-neurologic hospital patients; and the third (and second largest study) used non-demented well subjects. The study-specific ORs were 2.9, 3.1, and 3.0, while the combined OR was 3.0 (95% CI = [1.6 – 5.4], $p < 0.001$), with no confounder adjustments necessary. The occupational information was apparently primarily related to the last occupation, e.g., judge, high ranking military officer. A total of 386 cases and 575 controls was analyzed in these studies. 9.3% of the cases and 3.4% of the controls were judged to have had an occupation with likely medium or high ELF MF exposure. Among women, 31 (5.3%) were exposed to M/H occupational ELF MF, of whom 29 (95%) were seamstresses, who were classified as having high exposure based on measurements taken during the study. Seamstresses have subsequently been shown to have very high ELF MF exposures (e.g., Hansen *et al.*, 2000; Kelsh *et al.*, 2003; Szabó *et al.*, 2006).

D.3.2. Sobel *et al.* (1996b) and Davanipour *et al.* (2007) Studies – Positive Studies

These two studies utilized the databases of the nine (9) State of California funded Alzheimer's Disease Diagnosis and Treatment Centers (ADDTC). Sobel *et al.* (1996b), the second published study of occupational ELF MF and AD, used the Rancho Los Amigos (RLA) ADDTC database. There were 316 cases and 135 controls. Twelve percent (12%) of the cases and 5.3% of the controls had had a medium or high "primary" exposed (ELF MF) occupation. The Davanipour *et al.*, 2007) study used the databases of the other 8 ADDTCs. Seven and one-half percent (7.5%) of the cases and 3.8% of the controls had had a medium or high ELF MF "primary" occupation. Among the women in the RLA ADDTC study, 26 (8.4%) had M/H exposure, of whom 17 (65.4%) were seamstresses. In the Davanipour *et al.* study, among women, 50 (3.8%) had M/H ELF MF exposure, of whom 34 (68%) were seamstresses. This difference is statistically significant ($p < 0.001$). Among the men in the RLA ADDTC study, 14.8% had a medium or high ELF MF exposed occupation, while in the Davanipour *et al.* ADDTC study, 13.5% had a medium or high ELF MF exposed occupation. This difference is not significant. It thus appears that the women in the combined populations from which the ADDTCs in the Davanipour *et al.* study have drawn their patients have a lower rate of ELF MF exposed occupations than the population from which the RLA ADDTC draws its patients. This is not too surprising because Los Angeles has a large apparel manufacturing industry.

The OR (adjusted for age-at-onset, gender, and education) for medium or high ELF MF exposure in the RLA ADDTC study was 3.9 (95% CI = [1.5 – 10.6], p = 0.006). The ORs for medium or high ELF MF exposure in the Davanipour *et al.* ADDTC study were lower: 2.2 (p < 0.02; 95% CI = [1.2 – 3.9]) and 1.9 (p < 0.04; 95% CI = [1.04 – 3.6]), using age-at-exam and age-at-onset, respectively, plus gender and history of stroke in the model. These ORs are all statistically significant. In the two studies, the 95% CIs greatly overlap and, under the assumption of normality of the natural logarithms of the odds ratios estimators and a straightforward hypothesis test that the means of two independent normally distributed variables are equal, the null hypothesis that the corresponding ORs are equal cannot be rejected at the 0.05 level.

D.3.3. **Other AD/Dementia and Occupational ELF MF Exposure Studies**

Studies with (at least some) Positive Results

Qiu et al. (2004) *Study* Qiu *et al.* (2004) studied a Swedish cohort of 931 subjects, aged 75+ at baseline, followed for up to 7 years. Job history was usually obtained from the next-of-kin, but only after 4 years of follow-up. ELF MF exposure assessment was estimated using previous occupational exposure studies, specific measurements (e.g., seamstresses and tailors), and expert opinion. During the follow-up period, 265 subjects developed dementia, with 202 receiving an AD diagnosis. Numerical exposure estimates were obtained using both the longest held occupation, last occupation, and any occupation. The estimated average daily ELF MF exposure was used to classify individual exposure.

Exposure for a sample of seamstresses and tailors was measured at the head. They were classified as having low exposure. Exposures of seamstresses who used industrial sewing machines and workers who used home sewing machines likely were under estimated by Qiu *et al.* (2004): 5.5 mG for "industrial seamstresses" and 1.9 for tailors. Qui *et al.* only considered home sewing machines, which at the head had a mean exposure of 10 mG. For "industrial seamstresses, they assumed that 50% of the workday was at a 10 mG exposure and 50% was at background, 1 mG. This gives an average exposure of 5.5 mG. For tailors, they assumed that only 10% of the workday was spent sewing, so the mean exposure was 1.9 mG. There are several problems with this determination of exposure for seamstresses and tailors:

1. exposures to the head are among the lowest body exposures and are not necessarily the sole important exposure;
2. even in Sweden, it is unlikely that home sewing machines were exclusively used. It is more likely that most of the machines were industrial machines, which produce much higher fields constantly, even when sewing is not occurring;
3. seamstresses have exposure most of the workday;
4. ambient exposure levels in industrial settings have been measured at up to 6 mG (Sobel and Davanipour, unpublished Finnish data);
5. tailors would not make a living sewing only 0.8 hours per day.

Hansen *et al.* (2000) found that, at the side of the waist, mean full-shift exposure for industrial machines was approximately 30 mG, while Qiu used a figure of 10 mG. Based on unpublished measurements on AC home sewing machines, Sobel and Davanipour (1996c) found that exposures to the head were usually the lowest measurements, while the chest, pelvic area, thigh, knee, right arm and hand had much higher exposures (Table 8). In addition, foot pedals can produce high magnetic fields (Table 8). Also, AC/DC converters in the handles (right side) of computerized home sewing machines constantly produce high magnetic fields – about 75 mG at 2 inches away from the handle. The right hand, lower right arm, and knee regularly receive high exposures (Table 8). Thus, the 10% sewing time assumed by Qiu *et al.* (2004) does not mean that significant exposure is not over a longer time period. The biological plausibility of hypotheses discussed above provides an argument that exposure to other body parts may also be deleterious. The numbers or percentages of industrial seamstresses and/or home sewing machine workers were not provided by Qui *et al*. Note: seamstress' exposure assessment is discussed further in Section V.B.

Nevertheless, for the principal occupation, but not for the last occupation or cumulative lifetime exposure, Qiu *et al.* (2004) found statistically significant ORs: OR=2.3 (95% CI = [1.0 – 5.1]) for AD and OR=2.0 (95% CI = [1.1 – 3.7]) for any dementia for men with average exposures greater than 2 mG. For women, no increase in risk was found for the principal occupation, last occupation, and all occupations combined. The average lengths of time in the last and principal occupations were not provided. Thus, comparison with the Feychting *et al.* study (1998a) could not be made.

The proportions of subjects with at least 2 mG exposure were 28.2% for AD cases and 28.8% for controls for the principal occupation (Table 6). For all occupations combined, the proportions were higher. For men, with cases and controls combined, the proportions were 43.1% and 33.0%, respectively, for principal occupation and all occupations combined. For women, the proportions were 24.3% and 32.1%. In the Sobel *et al.* (1995, 1996b) and Davanipour *et al.* (2007) studies, the proportion of female cases and controls with medium or high exposure (considered above 2 mG) was only 5.5%, 80% of whom were seamstresses or had allied professions with significant ELF MF exposure, e.g., cutter. Thus, in these three publications, the exposure category for women contained a higher percentage of subjects with very high exposure. This may explain the lack of findings among women. The occupations which were in the exposure categories 'at least 2 mG' (dichotomized exposure) or 'at least 1.8 mG' (trichotomized) were not provided by Qiu *et al.*(2004).

Harmanci et al. (2003) Study Harmanci *et al.* (2003) conducted a cross-sectional, population-based study of Alzheimer's disease by selecting a random sample of 1067 subjects at least age 70, among whom 1019 (96%) agreed to participate in the study. AD was determined in a two-step process: a screening exam using the Turkish version of the Mini-Mental State Exam MMSE, followed by an expert clinical exam among those whose MMSE scored indicated cognitive impairment. Two hundred twenty three (223) were asked to have a clinical exam, and 155 (69.5%) agreed. Among the subjects with a "normal" score on the MMSE, 126 were randomly selected for a clinical examination. Among these 281 subjects, 57 were clinically diagnosed as having possible AD, and 127 were determined to be cognitively normal. These subjects were included in the case-control study. M/H ELF MF exposed occupations were stenographers and typists, carpenters and joiners, metal molders and core makers, tailors, dressmakers, and hatters. Except for stenographers, these occupations were considered to result in medium or high ELF MF exposure in the Sobel *et al.* (1995, 1996b) and current study. A stepwise backwards logistic regression analysis was used. Medium/high ELF MF exposure occupations had an adjusted OR of 4.0, with a 95% CI of [1.02 – 15.78]. It is interesting to note that use of electrical residential heating was also a risk factor (OR = 2.8, 95% CI = [1.1 – 6.9]).

Feychting et al. (1998a) Study In the case-control study by Feychting *et al.* (1998a), ELF MF exposure during the last occupation, but not during the longest held occupation, was a risk factor for dementia not caused by a single stroke. The last occupation was held an average of 24.8 years among cases and 25.9 and 25.1 years among subjects within the two control groups. Consequently exposure during the last occupation was over a significant period of time. Using the two control groups, the ORs for dementia were 3.3 and 3.8 with 95% CIs of [1.3 – 8.6] and [1.4 – 10.2] for occupations with geometric mean ELF MF exposures estimated to be at least 2 mG. Housewives were excluded from the analyses. The ORs for Alzheimer's disease were somewhat lower (2.4 and 2.7). When the analysis was restricted to subjects aged 75 and below at onset or examination, the ORs (5.0 and 4.8) for AD were statistically significant. Also, for subjects of all ages with occupations likely to have resulted in an average ELF MF exposure above 5 mG, the ORs for AD were both high, but significant for one referent group (OR = 8.3), and not for the other (OR = 4.1). The Feychting *et al.* study was small: 44 dementia cases had occupational data, 29 of whom were diagnosed with AD. 43% of the cases were in the ELF MF exposed group, while 23% and 19% of the controls were in this exposure group. Given these high percentages, it is clear that some lower ELF MF exposed occupations were classified in the exposed category than were classified in this study and the earlier Sobel *et al.* studies (1995, 1996b).

Chang et al. (2004) Study Chang et al. (2004) studied exposure to ELF MFs and other possible risk factors for AD among 62 AD patients and 124 controls, all of whom were elderly ex-military personnel, aged 66 to 102. (The published paper is in Chinese and we only have the PubMed English translation of the article's abstract.) Cases and controls were matched for age. Univariate and multivariate logistic regression models were analyzed. "Early" exposure to ELF MFs had an odds ratio of 2.49, with a 95% CI of (0.96-6.45).

Röösli et al. 2007 Study (Röösli et al. 2007) used records from the Swiss Federal Railway on employees who were employed or retired between January 1, 1972 and December 31, 2002. Employees in the following categories were used in analyses: train drivers, shunting yard engineers, train attendants, and station masters. "Average" ELF MF exposure for each year was assessed, based on measurements and "modeling". Five (5) ELF MF exposure indices were used: train drivers vs the other 4 occupations; cumulative work-time exposure (microtesla [μT] years); cumulative time above 10 μT; cumulative exposure up to 10 years prior to death or study closure; exposure within 20 years before death or study closure. Death certificates were used to determine disease status: AD (not coded in ICD-8 and only for subjects whose death was from 1995-2002); senile dementia (including AD); Parkinson's disease (PD); amyotrophic lateral sclerosis (ALS); cardiovascular disease (CVD); and respiratory tumor (RT). The total sample size for analysis was 20,141. Cox proportional hazards models were used to estimate the hazard ratio (HR) with station masters as the referent group. Station masters had, by far, the lowest ELF MF exposure.

Generally, train drivers experienced a very much higher ELF MF exposure than shunting yard engineers, train attendants, or station masters. ELF MF exposure was not associated with death due to (or with) CVD, PD, ALS, or RT. For senile dementia, which included AD, the HR for train drivers was 1.96, with a 95% CI of (0.98-3.92). For AD only, the HR was 3.15 with a 95% CI of (0.90-11.04). It should be noted that the number of deaths due to or with senile dementia or AD were small among the train drivers, shunting yard engineers, train attendants, and station masters, respectively: 30, 3, 17, 11 for senile dementia; 14, 2, 6, 3 for AD. This leads to wide confidence intervals.

Risks associated with increasing cumulative ELF MF exposure were assessed by determining hazard ratios related to exposure tertiles, with the lowest tertile as the referent group. There was an apparent possible increase in risk for subjects in the highest tertile, although the 95% CIs included 1.0.

Risks were also assessed by determining the HR for the number of years of exposure at or above 10 µT. In this analysis, risk increased by 5.7% for senile dementia and 9.4% for AD. Both figures are statistically significant at the 0.05 level: 95% CIs were above 1.0.

Studies with Only or Mostly Negative Results

Graves et al. (1999) Study Graves *et al.* (1999) studied 89 matched case-control pairs. Complete occupational histories were obtained. ELF MF exposure in a given occupation was defined as having at least "probable intermittent exposures (a few minutes)" above 3 mG. A high exposure category was defined as exposure of "1 to several hours" above 3 mG. Two industrial hygienists rated the occupations. Thus, many exposed subjects likely had a low average exposure. 19.1% and 21.4% of the cases were considered to have been 'ever' exposed, while 21.4% and 22.5% of the controls were considered 'ever' exposed. An unknown number of subjects, classified as having experienced ELF MF exposure, would not have been so classified in most or all of the other studies of neurodegenerative diseases or cancer. The estimated adjusted ORs for 'ever' having been exposed were 0.74 and 0.95, depending upon which industrial hygienist's classification was used (Graves *et al.*, 1999).

As noted above, the Feychting *et al.* (1998a) study found elevated odds ratios associated with the last occupation, and in the Sobel *et al.* studies (1995, 1996b) and the Davanipour *et al.* (2007) study, occupational information most likely related to the last occupation. Also, Feychting *et al.* (1998a) did not find an increased risk associated with measures which included earlier occupations, e.g., highest exposed occupation and longest held occupation. Qui *et al.* (2004) found elevated risk associated with the principal occupation for males. Consequently, 'ever' vs 'never' exposed, as used by Graves *et al.* (1999), may not be an appropriate comparison.

Graves *et al.* (1999) also used a cumulative exposure index, the weighted sum of the numbers of years in each occupation with the weights being 0, 1 and 2 for no exposure, only "intermittent exposures" above 3 mG, and exposure for "1 to several hours" above 3 mG, respectively. Using the non-zero cumulative index values, exposure was dichotomized at the median as 'low' or 'high'. Adjusted ORs for 'low' or 'high' cumulative exposure versus no exposure were also close to 1.0. The last or the primary occupation was not separately analyzed.

In summary, the non-significance of the ORs in the Graves *et al.* (1999) study may be due to three reasons: (1) less restrictive definitions of magnetic field exposure resulting in minimally exposed subjects being classified as having been 'ever exposed' or even highly exposed; (2) equal weight given to exposure during any age period, e.g., age 25-45 and age 45-65; (3) a cumulative exposure metric which equates what can be negligible exposure with significant exposure, e.g., negligible exposure for 20 years equals significant exposure for 10 years. In addition, there were no seamstresses among their subjects, who were from an HMO established primarily for union families. Seamstresses are seldom in a union.

Seidler et al. (2007) Seidler et al. (2007) conducted a case-control study by recruiting dementia-diagnosed cases, all 65 or older, from 23 general practices located in Frankfurt-on Main and neighboring cities. Recruitment was primarily based on the Mini-Mental State Examination. The Hachinski Ischemic Score was used in an attempt to differentiate between AD and vascular dementia (VaD). 195 cases (45 men and 150 women) were obtained: 108 were thought to have "possible" AD, 59 "possible" VaD, 25 had "secondary" dementia, and 3 an "unclassified" dementia. Imaging studies were also used for differential diagnostic purposes, if available. Population controls were randomly selected among those 65+ years of age who scored at least 27 on the MMSE. A second control group was selected from the general practices which contributed dementia cases. These controls needed to be ambulatory and also were required to have a MMSE of 27 or above. The authors state, but do not provide any other information, that "preliminary" analyses using the control groups separately produced "comparable results" with one exception: the ORs for blue collar work were "markedly" higher (p<0.1) for ambulatory controls than for population controls. Based on these unpublished analyses, the control groups were combined for "final" analyses. There were 229 controls in these latter analyses: 75 men and 154 women.

Analyses are conducted for dementia, possible AD, and possible VaD cases. However, the diagnostic methods used were really quite insufficient. For example, subjects with depression often have a low MMSE score.

Occupational histories were obtained by interview. Informational items obtained were job phase, job title, industry, and specific job tasks for every job that lasted at least one year. Next-of-kin were used for the dementia subjects, unless there was no next-of-kin and the subject was in the "first signs of dementia". These cases were not excluded in the published results because the results were not "fundamentally" different without them. Only jobs prior to the date of symptom onset or more than 4 years prior to dementia diagnosis if symptom onset timing was unknown were considered. Again, exclusion of these cases did not "substantially" alter the study results. The median time interval between the end of the last job and dementia diagnosis was 17 years for men and 24 years for women, while the for the controls the medians were 10 and 21 years, respectively.

Job titles were coded by experienced members the Frankfurt Institute for Occupational Medicine according to the Classification of the Federal Statistical Office in Germany and the Occupational Classification of the Finnish Censuses. Two-digit occupational codes were used. ELF MF exposure levels for each job were estimated by an "expert" co-author from the German Federal Institute for Occupational Safety and Health, blinded to case-control status. Exposure categories were specified as follows: < 1 mG; 1-2 mG; 2-10 mG, 10-100 mG,; 100-1000 mG, and > 1000 mG. (It is not clear in which category the lower and upper limits of each of the middle 4 categories belong.)

Analyses were based on cumulative exposure and maximum exposure to ELF MF, as determined by the expert co-author. ORs were determined for the 15 primary occupational two-digit categories (ever vs never worked in the category and per 10 years work) and for estimated cumulative exposure and maximum exposure. ORs were adjusted for age, region, gender, dementia in parents, and pack-years of smoking. The referent group consisted of subjects who never worked in the given category and who held white-collar jobs as their main occupation

Statistically significant findings among the ever vs never analyses were as follows:

Dementia Cases

- food & beverage processors; tobacco product makers - OR=4.1, 95% CI = (1.4 , 11.8);

- laborers (unskilled workers) – OR=7.6; 95% CI = (1.7 , 34.2);
- blue-collar work as the main occupation – OR=1.6; 95% CI = (1.0 , 2.5)

AD Cases

- blue-collar work as the main occupation – OR=1.7; 95% CI = (1.0 , 3.1)

VaD Cases

- food & beverage processors; tobacco product makers - OR=7.3, 95% CI = (2.0, 27.3);
- laborers (unskilled workers) – OR=6.3; 95% CI = (1.0 , 39.2).

Analyses based on "per 10 years" of work which were statistically significant or nearly so for possible AD were as follows:

- metal workers (machinery fitters, machine assemblers, mechanics, manufacturers of precision instruments, plumbers, welders, sheet metal and structural metal preparers and erectors – OR=2.2; 95% CI = (1.0 , 5.1),
- electrical and electronics workers – OR=2.7; 95% CI = (0.9 , 8.1),
- spinners, weavers, knitters, dyers, tailors, dressmakers – OR=1.4; 95% CI = (0.9 , 2.2),
- construction workers, including structural engineers, civil engineers) – OR=12.9; 95% CI = (0.9 , 186).

The "ever" versus "never" analyses are really quite inappropriate because the duration of time in the specific and general occupational categories can be quite low. The "per 10 years" analyses are thus more appropriate, but the sample sizes within job categories are quite small, except for "spinners, weavers, knitters, dyers, tailors, and dressmakers". However, it is not clear what the actual ELF MF exposures for spinners, weavers, knitters, and dyers might be.

The categories of (1) metal workers, (2) electrical and electronics workers, (3) spinners, weavers, knitters, dyers, tailors, and dressmakers; and (4), construction workers contain many of the occupations classified as medium or high ELF MF exposed occupations in the Sobel, Davanipour et al. papers and the papers by those who have essentially used the same classification methodology. One of the problems in the Seidel el al. (2007) paper is that the higher classification categories contain may occupations with low exposure.

The authors have available to them the actual specific occupations of each subject. They could therefore classify subject ELF MF exposure using the Sobel-Davanipour et al. methodology to reanalyze their data and determine if their findings for presumptive dementia (cognitive dysfunction) or AD patients replicate (or not) the Sobel, Davanipour et al. findings.

Andel et al. (2010) Study This study uses subjects from the Swedish Twin Registry. All subjects were 65 years or older in 1998. In all, 9,508 subjects had both a dementia/AD diagnostic workup and ELF MF occupational exposure estimates. 27.9% of the subjects were classified as having high exposure – above 2 mG. Among the subjects diagnosed as having dementia, 33.8% were classified as having had high exposure. The figure for subjects diagnosed with dementia was 34.0%. Among the controls, the corresponding figure was 27.8%. Dementia and AD were diagnosed in a structured, presumably appropriate manner : 216 (2.27%) with dementia; 141 (1.49%) with AD. Age at dementia onset (≤ 75 vs > 75) was determined by informants, presumably family members. Analyses were adjusted for covariates: gender, education, coronary disease, and stroke. Subjects were classified into three (3) exposure groups: < 1.2 mG, 1.2 to < 2.0 mG, and ≥ 2.0 mG. The referent group consisted of subjects with estimated exposure below 1.2 mG. Note that in the manuscript microTesla (μT) units were used: 1 mG = 0.1 μT. For all subjects, the dementia adjusted odds ratios (AORs) were 1.41 (p=0.079) for exposure between 1.2 and <2.0 mG and 1.38 (p=0.108) for exposure ≥ 2.0 mG. The AD AORs were 1.35 (p=0.211) and 1.38 (p=1.53). For age of onset ≤ 75, the AORs were 1.94 (p=0.03) and 2.01 (p=0.022) for all types of dementia and 1.69 (p=0.215) and 1.94 (p=0.090) for AD. For age of onset greater than 75, the AORs were much closer to 1.0 and clearly not significant. Analyses were conducted also for manual and non-manual workers separately. AORs for non-manual workers were clearly non-significant. For manual workers, the AORs for dementia and AD had p-values below 0.05, except for exposure ≥ 2.0 mG for AD when the p-value was 0.056.

It is our opinion that the ELF MF exposure assessment is not accurate in this study and other studies (e.g., breast cancer) which use the same exposure assessment methods and data. Specific occupational information was obtained by interview and then sent to "Statistics Sweden for coding according to categories from the 1980 Swedish Population and Housing Census". For men, occupational exposure assessment was based on measurements of a sample of 1098 Swedish men (Floderus et al., 1996). For women, the results of a study of 49 occupations by Forssén et al. (2004) have been used. This latter paper is also discussed below in our discussion of breast cancer, primarily in Section IV.E. We have two major concerns with the occupational classifications with respect to ELF MF exposure:

1. Generally, government classifications of occupation are wider than occupational determination based on individual subject information. Individual ELF MF exposure classification based on government classifications is therefore not likely to be particularly accurate. This will result in many individuals being misclassified as having exposures above 2 mG. The exposure classification methodology used by Davanipour, Sobel et al. and others has, we believe, much lower misclassification rates for 2.0 mG and above. For example in Davanipour et al. (2007) the rates of classification were 7.5% and 3.8% for AD cases and controls, respectively. As stated above, the comparable classification rate in the Andel et al. (2010) study was 27.9%.
2. The Forssén et al. (2004) measurements for women classified seamstresses as having low ELF MF exposure. This is very much out of line with our experience in Finland and in California and with the experiences of other researchers. Davanipour & Sobel measured ELF MF exposures in two clothing manufacturing companies in Finland. The ambient exposure, except during lunch time, among seamstresses and associated workers (e.g., cutters) in the same areas was over 6 mG. Exposures of individual seamstresses, all of whom used AC current industrial sewing machines, were much higher at every body location. We personally measured scores of seamstresses. The lowest exposure to any body part was 20 mG (e.g., Hansen et al., 2000). The usual work pattern was as follows: (1) the seamstress sits at a U-shaped table; (2) clothes to be sewed are folded on the right hand side; (3) the seamstress selects an article, sews it as specified; and (4) refolds the article, placing it on the left hand side of the desk.

All this time, the sewing machine is producing ELF MFs. This is because the motor is always on and a clutch needs to be engaged in order to move the needle. The seamstresses are doing this work for 6-8 hours per day. Seamstresses who work in drycleaners stores certainly do not sew all day long, so their exposure would be lower.

E. RF Exposure and Alzheimer's Disease

We found no human studies of AD and RF to discuss. The single published epidemiologic study of RF and melatonin is discussed in Section II (Burch *et al.*, 2002).

E.1. Transthyretin Studies

There have, however, been studies related to the effect RF exposure on transthyretin (TTR), also referred to as prealbumin. TTR is found in the brain, cerebrospinal fluid (CSF), and blood. Based on earlier research related to Aβ deposition (discussed below), Söderqvist et al. (2009a,b) investigated the effect(s) of RF on TTR in two studies. Söderqvist et al. (2010) discusses these same studies. In these studies, serum TTR levels are used as indicators for CSF and (presumably) brain TTR levels. However, there is apparently no study demonstrating that this assumption is valid.

1. In the 2009a study, 500 females and 500 males, aged 18-65, were randomly recruited from the municipality of Örebro, Sweden. Consenting subjects initially completed a questionnaire which included employment history, use of specific types of wireless telephones, X-ray, chemical, and radiation exposures (e.g., in medical therapy), and health and lifestyle questions, including physical exercise and disease history. An initial blood sample was collected from each subject as close to the end of a work week as possible. TTR concentrations (g/L) were determined using "standard immunoephelometric techniques". 133 (26.6%) of the male and 184 (36.8%) of the female subjects who were "recruited" fully participated. TTR assay results were log-transformed in all statistical analyses. Short-term wireless telephone use was determined by cumulative use (minutes) on the day the blood sample was delivered. Long-term use had two categories: "cumulative use" in total hours; and years since initial use. These short- and long-term figures were presumably guestimates by the study subjects. High TTR was chosen as the highest quartile (> 0.31 g/L. Low TTR was ≤ 0.31 g/L.

   There was no indication that wireless telephone use for at least 5 years or at least 10 years affected TTR levels as dichotomized. However, using the TTR levels themselves, for cumulative use, among men, there was an indication of increased risk with increasing use of mobile telephones (both analogue and digital). That is, the p-values were between 0.05 and 1.0. For years since first use, among men, the results were stronger. The p-values were below 0.05 for mobile telephones (all phones and analogue only). However, among men, for Universal Mobile Telecommunications System (UMTS) telephones there was declining risk with higher use (p=0.02).

   For short-term use, there were no findings of significance or, evidently, marginal significance, except in one instance. Among women, the shorter the time between last use of a mobile telephone and blood samples, the lower the TTR value (p-0.03).

There is no indication that the statistically significant or marginally significant finding have any biological importance.

2. Based on these short-term use finding, Söderqvist et al. conducted a "provocation" study, exposing volunteers to an 890 MHz mobile "phone-like" signal. Forty-four volunteers, aged 18-30 were recruited. Exposures occurred during the working day: 8 am – 5 pm. Exposures were over a 2 hour period, with blood samples collected prior to exposure, after a 30 minutes "rest" period, immediately following the provocation, and 60 minutes after the provocation. The provocation exposure had an average $kSAR_{1G}$ of 1.0 watts/kg. Seemingly the study design did not work out very well. The biggest mean change was a decrease between sample 1 and sample 2, when presumably nothing much was happening, except that the subjects were told to rest. The mean changes were very minimal between sample 2 and post-exposure samples 3 and 4, especially compared to the between subject values. There was also a control group who did not have any exposure. Their TTR measurements were not much different from the experimental groups measurements. However, no statistical comparison was presented.

   In short, this study seems to have provided no useful information.

The questions of importance here are (i) whether TTR concentrations in serum are indicative of concentrations in the CSF and brain and (ii) whether TTR inhibits or increases the aggregation and neurotoxicity of Aβ.

i. As mentioned above, we could find no studies of the relationship(s) between serum and CSF or brain levels of TTR.

ii. In *in vitro* studies, Schwarzman et al. (1994, 1996) found that CSF TTR binds to Aβ, possibly preventing or limiting amyloid formation within the brain. Their conclusion was that perhaps TTR helps prevent or delay AD onset. Serot et al. (1997) studied elderly AD patients and controls with ages between 2 and 90. TTR concentrations in CSF increased with age among the controls. TTR concentrations among the AD cases were similar to those controls in middle age and lower than the elderly controls (20.02 mg/l (sd=2.45) vs 17.49 mg/l (sd=2.02), $p<0.001$). The authors suggest that AD development may result in a lowering of TTR secretion. Lovell et al. (2008) studied the "aberrant" protein complex prostaglandin-d-synthase (PSD) and TTR in the CSF of autopsy verified late-onset AD patients, patients with mild cognitive impairment (MCI), and controls. They found that complexed PDS/TTR was significantly increased in the ventricular CSF of the AD and MCI patients compared to normal controls. This possibly explains the results of Serot et al. (1997). Animal and cell studies have found that TTR infusion leads to a reduction in Aβ deposits (Link, 1995), lack of neurodegeneration in the transgenic mouse AD model Tg2576 (Stein and Johnson, 2002), inhibition of Aβ aggregation, toxicity, and induced apoptotic changes in cultured cells (Giunta et al., 2005).

    Wati et al. (2009) then studied TTR and vascular Aβ deposition in two (2) transgenic mouse models of AD: $Tg2576/TTR^{-/-}$ which lacks endogenous TTR, but produces human variant amyloid precursor protein (APP), and $Tg2576/TTR^{+/-}$, which does not lack endogenous TTR. The $Tg2576/TTR^{-/-}$ mice had a significantly reduced Aβ burden compared to the $Tg2576/TTR^{+/-}$ mice, contrary to the researchers expectations. Their result indicates that, in their animal model, TTR appears to be associated with increased risk of amyloid burden.

On the other hand, using a different mouse model ceAPPswe/PSIΔE9/TTR$^{+/-}$ versus ceAPPswe/PSIΔE9/TTR$^{+/+}$, Choi et al. (2007) found that amyloid deposition in the hippocampus and cortex was elevated in the brains and "accelerated" in the hippocampus and cortex of the ceAPPswe/PSIΔE9/TTR$^{+/-}$ mice compared to the ceAPPswe/PSIΔE9/TTR$^{+/+}$.

Thus, results may be dependent upon differences between experimental species or sub-species. This suggests that (1) replication is warranted and (2) concentration on studies involving humans is appropriate if animal model replications continue to demonstrate differing results.

E.2. RF and Mitochondrial DNA (mtDNA) Oxidative Damage

Coskun et al. (2010) have demonstrated that mutations in the control region of mtDNA accumulate in the brain with age, with AD patients having a significant elevation of these mutations. These mutations in AD patients are associated with a reduced mtDNA copy number. They found that these mutations generally increase with age, both within the brain and in peripheral blood DNA and lymphoblastoid cell DNA. They argue that the mtDNA mutation level is inversely correlated with mtDNA copy number and positively correlated with beta-secretase activity, an indicator of increasing amyloid beta. Consequently, mtDNA damage may be associated with increased risk of AD.

Xu et al. (2010) studied oxidative damage to mitochondrial DNA related to 1800 MHz RF exposure in primary cultured cortical neurons. The neurons were exposed to 1800 MHz modulated by 217 Hz, using an average special absorption rate of 2 watts/kg for 24 hours. Examination of the neurons demonstrated a significant increase in 8-hydrodeoxyguanosine (8-oxodG), an indication of increased DNA damage. In addition, there was a clear reduction in the copy number of mtDNA and in the level of mtRNA after RF exposure. Xu et al. (2010) also conducted replicate assays, but with the addition of melatonin. The effects of RF exposure were reversed, but not completely.

IV. **BREAST CANCER**

Figure 2 provides a schematic outline of the areas of study providing evidence that ELF MF exposure can lead to breast cancer through an effect on melatonin production levels, and, of course, possible but unknown other pathways. Section references are provided in Figure 2.

There is now accumulating evidence that low melatonin production may increase the risk of breast cancer (BC). This evidence comes from *in vitro*, animal, and two longitudinal human studies. The *in vitro* and animal study literature is quite extensive, so only a highlight review is provided. There are numerous published case-control studies of residential and occupational ELF MF exposure as a risk factor for breast cancer. No epidemiologic studies of radiofrequency MF exposures and breast cancer have been published, which do not include ELF MF exposure, and which have reasonable data on RF exposure.

For a review of melatonin from basic research to cancer treatment, see Vjayalaxmi *et al.*, 2002.

- *Conclusion: There is sufficient evidence from in vitro and animal studies, from human biomarker studies, and from occupational and light at night studies to conclude that high ELF MF exposure may certainly be a risk factor for breast cancer. Most of the residential ELF MF exposure studies have been negative. This may be because "high" residential exposures are actually not very high. Individual exposures may be of importance, e.g., home sewing machines, hair dryers, AC/DC converters near the head of the bed, water pipes causing intermittent high exposures near living room or TV room sofas and easy chairs.*

As with Alzheimer's disease, we provide the results of a meta-analysis for breast cancer (Chen et al., 2010) despite our antipathy for such analyses, due primarily to varying study design components, exposure assessments, and subject differences. Chen et al. (2010) chose 15 studies published between 2000 and 2009. They found no associations between ELF MF exposure and (female) BC, including subgroup analyses based on exposure modes, menopausal status, and estrogen receptor status. These results are said to be in agreement with results by Erren (2001). Chen et al. (2010) found no statistically significant association between ELF MF exposure (residential, electric blanket, or occupational) and BC in general or BC based on menopausal status or ER status. There was substantial heterogeneity between studies. On the other hand, Erren (2001) found, using earlier studies not included in Chen et al. (2010), a slightly increased risk (referred to as RR) of BC in general: 1.12, 95% CI = (1.09 , 1.15). This is clearly statistically significant due to the very large sample size. Erren (2001) remarks that the results are quite variable between studies and "in part contradictory". He found that the primary methodologic problems were "probable misclassification of exposure" and "possible misclassification of the disease itself". Thus Chen et al.'s (2010) claims that (1) their results suggest no association between ELF MF exposure and BC and (2) are "in accordance" with Erren's results (2001) should be taken with a grain of salt.

A. *In Vitro* and Animal Studies Relating to Melatonin as a Protective Factor against Breast Cancer

A.1. *In Vitro* Studies Related to Prevention of Oxidative Damage; Comparative *in vivo* Studies with Vitamin C and Vitamin E

Melatonin has been found to neutralize hydroxyl radicals and to reduce oxidative damage in over 800 publications (Reiter et al., 1995; Tan *et al.*, 2002). Melatonin has also been shown to act synergistically with vitamin C, vitamin E and glutathione (Tan *et al.*, 2000) and stimulates the antioxidant enzymes superoxide dismutase, glutathione peroxidase and glutathione reductase (Reiter *et al.*, 2002).

- Using a cell-free system, Tan et al. and others have demonstrated that melatonin neutralizes hydroxyl radicals more efficiently than does reduced glutathione Tan *et al.*, 1993a; Bromme *et al.*, 2000).
- Melatonin reduces oxidative damage to macromolecules in the presence of free radicals (Reiter *et al.*, 1997, 2001a). One mode of action is as a free radical scavenger (Reiter *et al.*, 2001b).
- Melatonin increases the effectiveness of other antioxidants, e.g., superoxide dismutase, glutathione peroxidase, and catalase (Antolin *et al.*, 1996; Kotler *et al.*, 1998; Pablos *et al.*,

1995; Barlow-Walden *et al.*, 1995; Montilla *et al.*, 1997).

- Melatonin has protective effects against ultraviolet and ionizing radiation (e.g., Vijayalaxmi *et al.*, 1995). Vijayalaxmi et al. studied the effects of melatonin on radiation induced chromosomal damage in human peripheral blood lymphocytes (Vijayalaxmi *et al.*, 1996). Blood from human volunteers was collected before and after administration of a single 300 mg oral dose of melatonin. The post-administration samples of both serum and leukocytes had increased concentration of melatonin compared to the samples prior to melatonin administration. After gamma radiation and mitogen exposure, a sample of cells was cultured for 48-72 hours. Lymphocytes from the sample after melatonin was administered had significantly fewer chromosomal aberrations and micronuclei. Primary DNA damage was reduced. Vijayalaxmi et al. hypothesized that melatonin, in addition to its hydroxyl radical scavenging, may also stimulate or activate DNA repair processes (Vijayalaxmi *et al.*, 1998).

Melatonin has been found to be a more potent protector from oxidative injury than vitamin C or vitamin E (micromoles/kg) in several *in vivo* studies (for a review, see: Tan *et al.*, 2002). Melatonin was also found *in vitro* to scavenge peroxyl radicals more effectively than vitamin E, vitamin C or reduced glutathione (Pieri *et al.*, 1994; Reiter *et al.* 1995), although melatonin is not a very strong scavenger of peroxyl radicals (Reiter *et al.*, 2001b).

A.2. **Animal Studies of Mammary Tumor Prevention with Melatonin**

Several studies have found that melatonin inhibits the incidence of mammary tumors in laboratory animals either prone to such tumors or exposed to a carcinogen (e.g., Tamarkin *et al.*, 1981; Shah *et al.*, 1984; Kothari *et al.*, 1984; Subramanian and Kothari, 1991a,b; Blask *et al.*, 1991). In 1981, Tamarkin et al. found that supplemental melatonin, given on the same day as 7,12-dimethylbenz(alpha)-anthracene (DMBA) and continued for 90 days, lowered the incidence of mammary tumors from 79% in controls to 20% ($p<0.002$) in the melatonin treated Sprague-Dawley rats (Tamarkin *et al.*, 1981). When they treated pinealectomized rats with DMBA, the incidence of mammary tumors increased to 88%, indicating a possible effect on endogenous melatonin on tumor incidence. Similar results, but with somewhat different study designs, using female Holtzman rats given the carcinogen 9,10-dimethylbenzanthracene have been found (Shah *et al.*, 1984; Kothari *et al.*, 1984). Subramanian and Kothari studied the suppressive effect by melatonin in rats treated similarly with DMBA under varying light:dark schedules and time of melatonin administration in both intact and pinealectomized female Holtzman rats (Subramanian and Kothari, 1991a). They found that when administered during the initiation phase, melatonin only suppressed tumor development in intact animals. However, when administered during the promotion phase, melatonin had suppressive effects regardless of the presence or absence of the pineal gland. Subramanian and Kothari (1991b) also studied C3H/Jax mice and spontaneous mammary tumor development. Mammary tumors developed in 23.1% of mice provided with melatonin from 21 to 44 days of age, but in 62.5% of control mice ($p<0.02$). Furthermore, there was a decrease in serum 17-beta-estradiol levels in the melatonin treated mice ($p<0.05$). In a N-methyl-N-nitrosourea (NMU) model of hormone-responsive Sprague-Dawley rat mammary carcinogenesis, Blask *et al.* (1991) found that melatonin, given during the promotion phase, reduced the incidence of tumors and antagonized estradiol's stimulation of NMU-induced tumor incidence and growth. They, however, did not find a decrease in estradiol in the melatonin treated rats.

In two studies, Tan *et al.* (1993b, 1994) found that melatonin protected Sprague-Dawley rats from safrole induced liver DNA adduct formation. The protection was found at both physiological and pharmacological levels of supplementation. The level of protection was dose dependent. Intraperitoneal injection of paraquat causes lipid peroxidation, a decrease in total glutathione, and an increase in oxidized glutathione in Sprague-Dawley rats. Melchiorri et al. found that melatonin inhibits these effects (Melchiorri *et al.*, 1995). In addition, melatonin and retinoic acid appear to act synergistically in the chemoprevention of animal model tumors (Teplitzky *et al.*, 2001) and *in vitro* systems (e.g., Eck-Enriquez *et al.*, 2000).

A.3. Animal Studies Related to Prevention of Oxidative DNA Damage by Estradiol and Radiation

Karbownik *et al.* (2001) found that melatonin protects against DNA damage in the liver and kidney of male hamsters caused by estradiol treatment. They also found that in the testes, estradiol did not increase DNA damage, but that melatonin was protective against the natural level of oxidative DNA damage, as indicated by 8-hydrodeoxyguanosine (8-oxodG) levels. Several studies have found that laboratory animals are protected by melatonin from lethal doses of ionizing radiation (e.g., Blickenstaff *et al.*, 1994; Vijayalaxmi *et al.*, 1999; Karbownik *et al.*, 2000). Vijayalaxmi *et al.* (1999) and Karbownik *et al.* (2000) investigated markers of oxidative DNA damage and found that significant decreases in these markers in the melatonin treated animals.

A.4. Melatonin: Scavenger of $^{\bullet}OH$ and Other ROS

Melatonin is a powerful, endogenously produced scavenger of reactive oxygen species (ROS), particularly the hydroxyl radical ($^{\bullet}OH$). Other ROS which melatonin scavenges include hydrogen peroxide (H_2O_2), nitric oxide ($NO^{\bullet}$), peroxynitrite anion ($ONOO^-$), hypochlorous acid (HOCl), and singlet oxygen ($^1O_2$) (Reiter, 1991; Tan *et al.*, 2000, Hardeland *et al.*, 1995; Antolin *et al.*, 1997; Stasica *et al.*, 1998). $^{\bullet}OH$ is produced at high levels by natural aerobic activity. ROS are also produced by various biological activities or result from certain environmental and lifestyle (e.g., smoking) exposures.

Hydrogen peroxide does not appear to react directly with DNA (Halliwell, 1998), but does undergo chemical reactions within the cell nucleus which produce $^{\bullet}OH$, e.g., with Fe^{+2}. On the other hand, $^1O_2$ readily oxidizes the guanine base and causes HOCl, $ONOO^-$, and $NO^{\bullet}$ damage in various patterns (Halliwell, 1998).

However, $^{\bullet}OH$ is the most reactive and cytotoxic of the ROS (Halliwell *et al.*, 1986). $^{\bullet}OH$ appears not to be removed by antioxidative enzymes, but is only detoxified by certain direct radical scavengers (Tan *et al.*, 1999) such as melatonin.

Melatonin is found in every cell of the body and readily crosses the blood-brain barrier. It scavenges ROS at both physiologic and pharmacologic concentrations. In the literature, "physiologic" refers to blood level concentrations of melatonin, while "pharmacologic" indicates 2-3 orders of magnitude higher concentration. Recently, intracellular levels of melatonin, especially within the nucleus, have been shown to be naturally at "pharmacologic" levels for all cellular organelles studied to date (Maestroni, 1999; Reiter *et al.*, 2000).

Tan *et al.* (2002) review the underlying basis for melatonin's scavenging of ROS, which is briefly discussed here. From the known structure-activity relationships, the reactive center of the interaction between oxidants and the melatonin molecule is its indole moiety. This is due to its high resonance stability and quite low activation energy barrier towards free radical reactions. In addition, the methoxy and amide side chains contribute significantly to melatonin's antioxidant activity. The methoxy group in the C5 component of the molecule appears to prevent prooxidative activity. If this methoxy group is replaced by a hydroxyl group, under some *in vitro* conditions, melatonin may exhibit prooxidant capability. The mechanisms of melatonin's scavenging ROS appear to involve the donation of an electron to form a melatoninyl cation radical or a radical addition at site C3 of the melatonin molecule. (There are other possibilities also.) All known intermediates generated by the scavenging of a ROS by melatonin are also free radical scavengers. This is known (by some) as the 'free radical scavenging cascade reaction', which allows one melatonin molecule to scavenge 4 or more ROS. (See Tan *et al.*, 2007, for details).

A.5. Melatonin and Oxidatively Damaged Guanine in DNA

Davanipour et al. (2009) published the results of a study relating overnight melatonin production (as measured by aMT6s/creatinine levels in complete overnight urine samples) to the levels of oxidatively damaged guanine in DNA (as measured by urinary guanine damage/repair guanine products 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) and 8-oxo-7,8-dihydro-guanine (8-oxoGua). 8-oxodG is a product of the damage/repair of DNA guanine, while 8-oxoGua is a product of the damage/repair of either DNA or RNA guanine. Fifty-five (55) mother-father-oldest adult daughter families were recruited. All were healthy for their age. The age ranges were as follows: mothers – 43-80; fathers – 46-81; daughters – 18-51. The results were as follows:

- with or without adjustment for BMI or weight, among the mothers there was an inverse relationship between creatinine-adjusted aMT6s and 8-oxodG (p=0.02);
- among the mothers older than the oldest daughter (age 51.6) the significance level of the inverse relationship between creatinine-adjusted aMT6s and 8-oxodG fell to 0.009;
- among the fathers older than the oldest daughter, the inverse relationship between 8-oxoGua and creatinine-adjusted aMT6s was significant at the 0.03 level;
- among the oldest daughters, there was an increase in 8-oxoGua with increasing age.

This study appears to be the only research published to date on the relationship between melatonin production and DNA damage/repair in humans.

B. Longitudinal Human Studies of Low Overnight Melatonin Production as a Risk Factor for Breast Cancer

Conclusion: *Five longitudinal studies have now been conducted of low melatonin production as a risk factor for breast cancer. Two of the studies collected urine samples in an optimal manner to estimate the important component of melatonin production – overnight production. However, two (2) used first morning void, which is close to optimal and one (1) had to use 24-hour collection, which hides possible non-circadian rhythm, which can be deleterious. One study, which used first morning void urine, was limited to premenopausal BC. The study which used 24-hour urine samples was negative. Of the remaining 4 studies, three were positive and the one limited to premenopausal BC was problematic, perhaps due to lag times and the likely adverse effect of BC in its very early stage on melatonin production.*

> *Thus, there is increasingly strong longitudinal evidence that low melatonin production is a risk factor for at least post-menopausal breast cancer.*

There have been five (5) longitudinal studies, two of which were from the Nurses' Health Study cohort, of low melatonin production as a risk factor for breast cancer. Note that many breast cancers are associated with a decrease in melatonin production (Bartsch *et al.*, 1997). There is often a "rebound" after excision of the tumor, but it is not known if post-excision melatonin production is near the pre-tumor production level (Bartsch *et al.*, 1997). Thus, as with AD, it is not appropriate to use post-tumor melatonin levels in a case-control study of low melatonin as a risk factor for breast cancer.

DNA damage is the pathway through which normal cells become malignant. Thus, the greater the amount of DNA, the greater the probabilities of a malignant transformation and the development of cancer. Davanipour *et al.* (2009) have conducted a study on the association between endogenous melatonin levels and oxidative guanine DNA damage among mothers and their oldest sampled daughters. The mothers' age range was 43-80, while the oldest daughter's age range was 18-51. Nearly all of the mothers, but few of the daughters were postmenopausal. Complete overnight urine samples were obtained. Creatinine-adjusted aMT6s and 6- hydrodeoxyguanosine (8-oxodG) were assayed. 8-oxodG is a measure of the level of oxidative DNA damage. Creatinine-adjustment is not necessary because the 8-oxodG level using complete overnight urine is a measure of the total repair of oxidized DNA guanine during the night. There was a statistically significant (p=0.02) inverse association between the level of nocturnal melatonin production (aMT6s/creatinine) and 8-oxodG for the mothers, but not for the daughters. Statistical adjustment was made for age and weight; however, there was little difference in the results with or without adjustment. The correlation between creatinine-adjusted aMT6s and 8- oxodG was 0.35 (p=0.01).

Positive Studies

Schernhammer and Hankinson (2005) reported on the association between urinary melatonin levels and breast cancer risk in the Nurses' Health Study II. The study had collected first morning void urine samples prior to the diagnosis of any cancer in a sub-sample of the women in the study. Assays of aMT6s and creatinine for 147 women who developed invasive breast cancer, and 291 age-matched controls, plus 43 women who developed in situ breast cancer and 85 matched controls were analyzed. Analyses were based on quartiles of creatinine-adjusted aMT6s developed from the control data, with subjects in the lowest quartile as the referent group. (Thus, the analyses were conducted with a view that higher levels of melatonin production might be protective.) Unadjusted analyses, estradiol level adjusted analyses, and analyses adjusted for age-at-menarche, parity, age-at-first birth, family history of BC and benign breast disease, alcohol use, antidepressant use, and body mass index were conducted. It should be noted that low levels of melatonin are causally associated with earlier age-at-menarche (e.g., Cohen *et al.*, 1978; Sizonenko, 1987). Thus, inclusion of age-at-menarche in the adjustment is perhaps not appropriate. Analyses of cases and controls from the lowest and the highest quartile were statistically significant for each level of adjustment. The odds ratios (OR) were all 0.59. (In terms of risk associated with low melatonin production, the OR was 1/0.59 = 1.69.) Inclusion of the the cases with in situ breast cancer led to OR between 0.68 and 0.70. Significance levels were not provided. However, the 95% CI's for invasive breast cancer did not contain 1.0, while the 95% CIs when in situ breast cancer cases were included just barely contained 1.0.

In 2008, Schernhammer and Hankinson used the Hormones and Diet in the Etiology of Breast Cancer Risk (ORDET) cohort to study low overnight melatonin production as a possible risk factor for postmenopausal breast cancer. The ORDET study was conducted in northern Italy and included 10,786 healthy women aged 35-69 at baseline, 3966 of whom were postmenopausal. Complete 12-hour overnight urine samples were obtained. There were 178 subjects who developed postmenopausal BC prior to the Schernhammer et al. study analysis and met inclusion criteria, e.g., BC as the initial cancer, urine sample availability. Seven hundred ten (710) women were selected as controls, matched on age at enrollment (± 3 years), date of recruitment (± 180 days) and laboratory assay batch. Conditional regression models were used for analyses, adjusting for thirteen (13) known BC risk factors and circulating testosterone, which was a BC risk factor in the ORDET study. Analyses were performed using both aMT6s and creatinine-adjusted aMT6s. Analyses were done by quartiles of aMT6s. 95% CIs and trend p-values were calculated. Trend p-values were 0.05 or below when the analyses excluded in situ BC and below 0.10 when in situ BC was included. When analyses were conducted without current smokers, the trend p-values were below 0.005. Comparing the highest versus lowest quartile of aMT6s, the p-values were at or below 0.05 for invasive BC, including or excluding testosterone. When only non-current smokers were analyzed, the p-values were smaller. (Note: only 95% CIs were actually published.) Results were similar for creatinine-adjusted aMT6s analyses.

In 2009, Schernhammer and Hankinson used to Nurses' Health Study cohort to further investigate the relationship between urinary melatonin levels and postmenopausal BC. Spot morning urine assays for aMT6s were available for 357 postmenopausal women who developed incident BC after recruitment into the cohort and 533 matched controls. The analysis methods were much the same as in the previous paper. Quartiles of aMT6s among the controls were analyzed. In multi-variable adjusted analyses, the subjects in the lowest quartile of aMT6s had an increased risk ($p < 0.05$) of developing BC compared to subjects in the highest quartile. This was true for all BC, for in situ BC only, and for invasive BC only. Subjects in the lowest quartile also had an increased risk compared to subjects in the 3rd (highest) quartile for all BCs and for in situ BC only. Trend p-values were below 0.05 for all three groups: all BCs, invasive BC, in situ BC.

** It should be noted that the first morning void, especially when the subject has had urine voids during sleep time, is not as good as complete overnight urine collection in estimating nocturnal melatonin production. **

Negative Study

Travis *et al.* (2004) conducted a study of melatonin and breast cancer using the Island of Guernsey or Guernsey III longitudinal study. This study recruited women for an eight and one-half year period, ending in 1985. During the follow-up period, 127 women developed breast cancer. Three hundred fifty three (353) controls were selected with matching based on age, recruitment date, menopausal status, day of menstrual cycle (if applicable) when the urine sample was obtained, and number of years post-menopausal (if applicable). Twenty-four (24) hour urine samples were collected. These samples were evidently not divided between overnight and other time-of-day sub-samples. None of the analyses (all cases-controls, only pre-menopausal cases-controls, or only post-menopausal cases-controls) showed any hint of an increase risk associated with low 24-hour melatonin production.

** It is unfortunate that the 24-hour urine samples were not subdivided by time of day. It is the nocturnal blood level of melatonin that is important. About 85%-90% of pineal melatonin is produced nocturnally. The circadian rhythm appears to be vital for the effects of melatonin in regulation of important biologic functions, including immune response. This particular problem with the study makes the results suspect. (See Hrushesky and Blask, 2004, for further details.) **

Problematic/Peculiar Study

In 2010, Schernhammer et al. used the ORDET cohort to investigate premenopausal BC. There were 180 premenopausal BC cases, with 683 controls selected – nearly 4 per case – using the same matching criteria as was previously used. The urine samples were 12 hour, overnight (7:00 pm – 7:00 am) samples. There was a statistically significant trend towards **increasing risk** with higher baseline aMT6s. This was the opposite of what was likely anticipated. However, when current smokers were excluded, the increasing risk completely disappeared. On the other hand, among non-current smokers, a BC diagnosis within 3 years of urine collection was much more likely for subjects in the highest aMT6s quartile compared to subjects in the lowest quartile. Lag time from urine collection to BC diagnosis was also investigated among non-current smokers. Only after 8 years of lag time was there a statistically significant difference between the lowest and highest quartiles of aMT6s: an increase in risk associated with low production. Thus, this study's results are clearly perplexing. The authors recognize this and suggest that perhaps very early BC is causing an increase in melatonin production.

C. No Case-Control Studies of Low Melatonin Production as a Risk Factor for Breast Cancer

As mentioned previously, breast cancer itself often causes a decrease in melatonin production, e.g., Bartsch *et al.* (1997). It is therefore inappropriate to use current levels of melatonin production of breast cancer cases in a case-control study of whether low levels of melatonin are a risk factor for breast cancer, and none have been published.

D. Light-at-Night and Night Shift Work Studies as a Risk Factor for Breast Cancer – Surrogates for Low Melatonin Production

Conclusion: There is moderately strong evidence that both long-term light-at-night and night shift work increase the risk of breast cancer. Five (5) studies are reviewed, 4 of which are positive. The negative study did find an increased risk for light-at-night, but not shift work. This study classified subjects as having had rather short shift work as exposed. Only very few subjects had at least 8 years of shift work: 8 (1.6%) of cases and 19 (3.7%) of controls.

Several studies have found an increase in risk of breast cancer among women who have rotating night shift work or who otherwise experience light at night. Light at night (LAN) is well-known to cause a decrease in nocturnal melatonin production (e.g., Lewy *et al.*, 1980; Lowden *et al.*, 2004; Schernhammer *et al.*, 2004). Note that occupational studies of ELF MF exposure

(Section E, below) have included jobs with night shift work, e.g., flight attendant and radio/telegraph operators.

Positive Studies

- Lie *et al.* (2006) studied the occurrence of breast cancer among Norwegian nurses. All data were obtained from government registers. Among a cohort 44,835 nurses, who graduated from a 3-year nursing program between 1914 and 1980 and who were alive on January 1, 1953, or born after this date, 537 breast cancer cases which occurred between 1960 and 1982 were identified. (1960 was chosen because that was the first year for which fertility data were available.) Four (4) controls, alive and cancer free, for each case were selected from the nurse cohort, matched by year of birth (± 1 year). Controls were required to have graduated or started their initial job no later than the year the corresponding case was diagnosed with BC. Number of years of night shift work was estimated from work history and work locations. Statistical adjustments in OR estimates included total employment time and parity. The OR for 30+ years of night shift employment versus 0 years, was 2.21 ($p<0.05$), 95% CI = [1.10 – 4.45]. The p-value for trend was 0.01. When the analysis was limited to nurses aged 50+, the OR was 2.01 ($p>0.05$), 95% CI = [0.95 – 4.26]. The number of cases without night shift work was only 50 for all ages, and was 29 for nurses over age 50. The number of cases with at least 30 years of night shift work was 24. (No case below age 50 had 30+ years of night shift work.)

- Schernhammer *et al.* (2001) examined rotating night shift work as a possible risk factor for breast cancer in the Nurses' Health Study. The total number of years in which a subject had worked rotating night shifts of at least 3 nights per month was obtained in 1988. The sample was quite large: 31,761 nurses had not had any years meeting the night shift criterion; 40,993 had had 1-14 years; 4,426 had had 15-29 years; and 1,382 had had 30+ years. During the following 10 year period, 2,441 incident cases of breast cancer were identified. Compared to nurses who had had no qualifying years, the adjusted relative risk (RR) for nurses with 30+ years of rotating night shift work was 1.36, with a 95% CI of [1.04 – 1.78]. All subjects with 30+ of rotating night shift work were post-menopausal Analyses were also conducted within pre- and post-menopausal groups. The RR and 95% CI were the same for 30+ years of exposure, because the number of nurses with no exposure decreased slightly (from 925 down to 801). While not statistically significant, perhaps due to sample size, pre-menopausal nurses who had at least 15 years of shift work had an adjusted RR of 1.34, 95% CI = [0.77 – 2.33], essentially the same RR as post-menopausal women (RR=1.36, 95% CI = [1.04 – 1.78]) who worked night shift for at least 30 years. There were only 14 pre-menopausal nurses with 15+ years of exposure. The trend in RR for increasing years of exposure was statistically significant for post-menopausal nurses and all nurses. Adjustments were made for age, weight change between age 18 and menopause, and many other variables associated with breast cancer. The increase in risk was almost totally due to hormone-receptor positive breast cancers. This was the first prospective night shift and breast cancer study.

- Davis *et al.* (2001b) studied 813 breast cancer patients, aged 20-74, and 793 controls. The controls were obtained through random digit dialing and were frequency matched by 5-year age intervals. Lifetime occupational history, bedroom lighting, and sleep habits were obtained by interview for the 10 years prior to diagnosis. Not sleeping during nocturnal periods (when melatonin production is usually at its peak) had an OR of 1.14 for each night per week. The 95% CI was [1.01 – 1.28]. Night shift work had an OR of 1.6, 95% CI = [1.0 – 2.5]. There was a significant upward trend (p = 0.02) in the OR with increasing years and more hours per week in night shifts. Statistical adjustments were made for parity, family history of BC, oral contraceptive use (ever), and recent (but discontinued) use of hormone replacement therapy.

- Hansen (2001) studied BC risk among younger Danish women whose work was mostly at night. All women born between 1935 and 1959, and 30-54 years of age, were identified though the Danish Cancer Registry. The number of such women was 7,565. One control per case was randomly selected from the Danish Central Population Registry. Controls were (i) living, (ii) apparently cancer free, and (iii) working before the date of diagnosis of the corresponding case. Work history was obtained from the Danish pension fund database. No work history was found for 530 cases, so the number of case-control pairs for the study was 7,035. Using a national survey (1976) of women and working conditions, 4 occupational categories were identified in which at least 60% of the female employees so some work at night. These were manufacturing of beverages, land transport services, catering, and air transport services. For hospitals, furniture manufacturing, water transport services, and cleaning services, between 40% and 59% of the women work some night shifts. Comparisons were made between occupations in which 60%+ of the women work night shifts and occupations in which less than 40% work night shifts. Only occupations within 5 years of diagnosis were considered. This limit was based on suspected induction time for breast cancer. To be placed in the "exposed" category a women had to have worked at least 6 months in a night shift occupation. Statistical adjustments were made for age, social class, ages at birth of first and last child, and parity. The OR for all "exposed" occupations was statistically significant (p<0.05): OR=1.5, 95% CI = [1.3 – 1.7]. For women who worked at least 6 years in "exposed" occupations, the OR was 1.7 (p<0.05). The results were essentially driven by the catering and air transport service occupations. (It should be noted that these two occupations may also result in higher ELF MF exposure, compared to manufacture of beverages and land transport services.) The authors state that "(w)hen the 5-year induction time was ignored, the ORT decreased marginally".

Negative Study

- O'Leary *et al.* (2006) studied night shift work, light-at-night and BC in Long Island, NY, as part of the Electromagnetic Fields and Breast Cancer on Long Island Study (EFBCLIS) Group. There were 487 cases and 509 population-based controls, frequency matched to the expected age distribution of the cases in the study. These subjects had to have participated in the earlier Long Island Breast Cancer Study Project (LIBCSP). Each case had to have lived in the same home for at least 15 years prior to the diagnosis of breast cancer, while each control had to have lived in the same residence for at least 15 years prior to recruitment. Cases had to have had their BC diagnosis within the 12 month period beginning August 1, 1996. Controls were concurrently recruited. The LIBCSP had collected, via direct interview, complete job history information, including shift work – all jobs held for at least 6 months beginning at age 16, full time or part-time. The EFBCLIS repeated the job history interview, without the shift work information, for the period 15 years prior to the date of BC diagnosis (cases) or recruitment (controls). Military assignments were included. Light-at-night information was obtained by interview, and included information about sleep hours, frequency and length of having lights on during sleep time for the 5 year period prior to the reference date.

Exposure to shift work was defined as ever having had a job (≥ 6 months, either part or full time) with at least 1 day per week of shift work, during the 15 years prior to the reference date. Sub-groups were defined as follows: ever had an evening shift job; ever had an overnight shift job; ever had an evening shift, but never an overnight job; ever had an overnight shift; but never an even shift job. Statistical analyses were adjusted for reference date, parity, family history of BC, education, history of benign breast disease.

For any of the various categories of shift work during the 15 years prior to the reference date, there was no elevated risk of BC. However, 'any overnight shift work' had a statistically significant OR below one. The referent group included subjects with a jobs having less than 1 shift work day per week. Such a job could have been held for many years. The OR for at least 8 years of overnight shift work was statistically significantly below 1. For light-at-night within 5 years prior to the reference date, the only statistically significant finding was an OR = 1.65 for waking up and turning on lights at least 2 times per night versus doing so no more than 3 times per month.

The authors conclude that their study "provides mixed evidence for the light-at-night hypothesis". Analyses of shift work within 5 years of the reference date, the "induction" period used by Hansen (2001), were not presented. Overnight shift work was in the work history of only 26 cases and 50 controls; a duration of at least 8 years of overnight shift work was experienced by only 6 cases and 19 controls. Thus, the effective, "exposed" sample size was quite small. Information as to when this shift work occurred relative to the reference date was not provided.

E. Occupational Case-Control Studies of ELF MF Exposure as a Risk Factor for Breast Cancer

Conclusion: There is rather strong evidence from case-control studies that long-term, high occupational exposure to ELF magnetic fields is a risk factor for breast cancer. Six (6) independent studies are reviewed. Four (4) have positive conclusions, while two (2) are negative. The latest study is particularly strong. The two negative studies have serious shortcomings in exposure classification and come from the same research group.

There have been several case-control studies of occupations with more or less high ELF MF exposure and the risk of breast cancer. These studies have been generally positive, in the sense that there appears to be an increased risk. Earlier studies generally lack appropriate exposure information (e.g., Wertheimer and Leeper, 1994).

Positive Studies

- Peplonska *et al.* (2007) have conducted a large, population-based, case-control study of breast cancer and 73 occupational categories. All incident cases of cytologically or histologically confirmed breast cancer among women aged 20-74 in Warsaw and Lódź, Poland, in 2000-2002 were identified. 2,502 controls were randomly selected using the Polish Electronic System of Population Evidence, which maintains records on all citizens of Poland. Controls were matched to cases by city of residence and age ± 5years. A structured questionnaire was completed by 79% of the cases and 69% of the controls. The questionnaire included items related to demographics, reproductive and menstrual history, hormone use history, physical activity, occupational history for all jobs held at least 6 months, smoking, alcohol use, diet, cancer history in female relatives, medical and screening history, prenatal exposures, and history of weight and height development. Occupational information included job title, start and stop dates, employer, company products and/or services, work activities and duties, physical activity related to work, passive smoking, and exposures to a list of chemicals. The study was funded by the U.S. National Cancer Institute (NCI) and managed by Westat (Rockville, MD).

Statistical adjustment was made for age, age-at-menarche (≤ 12; 13-14; ≥ 15), menopausal status; age-at-menopause, parity ≤ 1; 2; ≥ 3), body mass index (< 25; 25-30; ≥ 30 kg/m^2), first degree female family history of BC, education (< high school; high school; some college or professional training; college degree), previous mammographic screening, and city of residence. Oral contraceptive use, marital status, tobacco and alcohol use, age-at-first full term birth, breastfeeding, recreational and occupational history were not used for adjustment in the final analyses because they had "little impact" on the results.

In the primary analyses, for each specific job category/industry, the referent group consisted of all subjects who did not work in that job/industry for at least 6 months. For each specific "white-collar" occupation, additional analyses using all other white-collar jobs as the referent group were conducted. This was thought to provide at least a partial account for socio-economic factors not accounted for by education. Similar blue-collar job analyses were not conducted. Several job categories containing occupations with elevated ELF MF exposure had statistically significantly elevated ORs.

** These ORs were significantly elevated despite the fact that all other occupations with elevated ELF MF exposure were placed in the referent group. **

ELF MF exposure was determined using a job exposure matrix developed within NCI for a brain cancer study. No, low, medium and high categories were developed by "experienced industrial hygienists". (No reference was provided.) The highest ELF MF exposure category of all jobs for an individual was used in analyses. 99% of the high exposed subjects were so ranked due to employment as machine operators and tenders in the textile apparel and furnishing industry. Information on which occupations were classified as low or medium ELF MF exposure were not provided.

** It should be noted that (1) 'tenders' generally provide maintenance to machinery and (2) operators of machines other than sewing machines, e.g., cutters, both have lower ELF MF exposure than seamstresses. **

The OR for high ELF MF exposure versus no exposure was significant: OR = 1.5, 95% CI = [1.1 – 2.0]. For low exposure, the OR was also significant: OR = 1.2, 95% CI = [1.0 – 1.5]. For medium exposure the OR was also 1.2, but the 95% CI was [0.9 – 1.5]. Additional data analyses were not provided. The OR for high exposure among textile apparel machine operators and tenders is in line with the statistically significantly increased OR for seamstresses in the Forssén *et al.* (2005) study (see below under "negative studies") discussed below. In the Forssén *et al.* study (2004), seamstresses were classified as having medium-low ELF MF exposure.

Specific ORs for occupations classified (surprisingly and for some likely incorrectly) as having high (as opposed to low or at most medium) ELF MF exposure by Forssén *et al.* (2004) (see below) were calculated: cooks (OR=1.0); computer scientists (OR=1.3); computer and peripheral equipment operators (OR=0.7); data entry keyers (OR=0.3); dentists (OR=0.6); dental nurses (OR=1.0); counter clerks and cashiers (OR=1.1); and telephone operators (OR=0.9).

- Labréche *et al.* (2003) studied occupational ELF MF exposure and post-menopausal breast cancer. Cases and controls were identified through pathology department records at 18 hospitals in Montreal, Canada. These hospitals treat most of the breast cancer cases in the area. Age was restricted to 50-75 at the time of initial diagnosis of primary BC. Cases had to be residents of the region and the diagnosis had to have been in 1996 or 1997. Controls had one of 32 other cancer diagnoses and were frequency matched by age and hospital. The following cancers were excluded: liver, intrahepatic bile duct, pancreas, lung, bronchus, trachea, brain, central nervous system, leukemia, lymphoma, and non-melanoma skin cancer, but not gastrointestinal (Schernhammer *et al.*, 2003) or colorectal cancer (Bubenik, 2001).

  Complete occupational history, including task descriptions, and other personal information was obtained by personal interview, either of the subject or a surrogate if the subject was deceased or otherwise unavailable. Specialized occupational questionnaires were used for specific occupations, including sewing machine operators, cooks and nurses. The development of these questionnaires was led by Jack Siemiatycki. See, for example, Siemiatycki *et al.* (1991, 1997). ELF MF exposures were estimated from detailed descriptions of tasks, equipment used, and the work environment by industrial hygienists intimately familiar with Montreal workplaces. The ELF MF exposure categories and primary occupations were as follows: no exposure (< 2 mG; low exposure (2-5 mG, "typical jobs", including VDT operators, electric typewriter operators); medium exposure (5-10 mG; denturists, machinists); and high exposure (≥ 10 mG; sewing machine operators, textile workers). The industrial hygienists "confidence" in each subject's exposure assessment was obtained as definitely no exposure, or low, medium, and high confidence of exposure.

  Exposures to benzene, perchloroethylene, and alphatic aldehyes, chemicals found in the textile industry, were also considered.

  Statistical adjustments were made for age at diagnosis, family history of breast cancer, education, ethnicity, age-at-bilateral oophorectomy, age-at-menarche, age-at-first full-term pregnancy, oral contraception use, duration of HRT, total duration of breast feeding, alcohol use, smoking, and body mass index, as appropriate. Adjustment was also made for proxy versus personal responses because proxies tend to report fewer jobs. In addition, duration of employment in the textile industry was an adjustment variable. As mentioned previously, adjustment for age-at-menarche is probably not appropriate due to melatonin's causal relationship with age-at-menarche.

In addition to the categorical analyses, the number of hours of medium or high exposure was used as a risk factor. The number of hours from the lower limit of the second quartile to the upper limit of the third quartile of medium/high exposure was 6000 hours. ORs were presented for a difference of 6000 hours.

All analyses, e.g., no exposure vs ever exposed, prior to 10 years before diagnosis, or before age 35, were non-signficant and non-elevated except for the following ones, adjusted for textile industry employment and other factors:

- ✓ No exposure vs medium-to-high exposure – OR = 1.90, 95% CI = [0.99 – 3.85];
- ✓ 6000 hour increase in medium-to-high exposure – OR = 1.21, 95% CI = [0.97 – 1.49];
- ✓ 6000 hour increase in medium-to-high exposure prior to 10 years before diagnosis – OR = 1.31 ($p<0.05$);
- ✓ 6000 hour increase in medium-to-high exposure prior to age 35 – OR = 1.54 ($p<0.05$).

The significant results appear to be primarily due to ELF MF association with progesterone positive and/or estrogen positive breast cancers.

The use of a 10 year lag eliminates exposure periods which may be too near the diagnosis time to be etiologically relevant. The analysis of exposures prior to age 35 identifies the time period when the development of female breast cells appears to cease.

The use of textile industry employment (yes/no) or length of time in the textile industry, as appropriate, as a covariate provides some adjustment for chemical exposures. Thus, the increase in the ORs when adjustment was also made for textile industry employment relates to ELF MF exposure.

Finally, controls also had cancer. While many of the excluded cancers may conceivably have ELF MF as a risk factor, some of the non-excluded ones may also. This is especially true if the melatonin hypothesis is correct. Thus, the OR estimates may be biased towards 1.

- Kliukiene *et al.* (1999, 2003, 2004) and Tynes *et al.* (1996) studied occupational ELF MF exposure and breast cancer among Norwegian women in general and radio and telegraph operators in particular. These were follow-up studies. A population-based cohort of 1.1 million women was developed using the 1960, 1970, and 1980 censuses. All women were working at the time of enrollment and had a potential for occupational ELF MF exposure. The follow-up period was from 1961-1992. Date of birth, and census information about occupation and socioeconomic status was obtained. Incidence of breast cancer was obtained from the Cancer Register of Norway. Out-migration information was obtained.

For the countrywide, all occupations study (1999), ELF MF occupational exposure assessment was not optimal, but was as follows. The first method used expert opinion. An expert panel, using written guidelines, decided whether a given occupation had ELF MF exposure above 1 mG for than 4 hours per week, between 4 and 24 hours per week, or more than 24 hours per week. Occupations were identified by a 3-5 digit industry code and a 3-digit occupation code. For cumulative exposure, the mean of each of the three (3) levels of exposure were used: 2 hours; 14 hours, 32 hours (based on a 40 hour week). It was assumed that each subject was in the same occupation from census to census, unless she died, emigrated or turned age 65.

The second method used the Swedish job exposure matrix used in the Forssén *et al.* (2000) study (below), which was constructed from observations of male workers. Cumulative exposure was categorized as below 9 mG-years, between 9 and 14 mG-years, between 14 and 30 mG-years, and above 30 mG-years. Exposure was also classified by number of work hours of exposure above background (1 mG): below 900 hours; 900-999 hours; 1000-1999 hours; 2000 or more hours.

Poisson regression, with adjustment for age, time period, and socioeconomic status, was used to estimate the relative risk (RR) of breast cancer. 22,543 breast cancer cases were diagnosed during the follow-up period. In the total cohort and the two sub-cohorts for those below or at least 50 years of age at inclusion in the cohort (Kliukiene *et al.*, 2004), the RRs were statistically significantly above 1.0 for each category of number of exposed hours, with below 900 hours as the reference category. For each cumulative exposure category above the reference category (below 9 mG-years, the RR for the total was statistically elevated. For the two sub-cohorts, the RRs were significantly elevated for the 9–14 and 14–30 mG-years categories. For the 30+ mG-years category the RRs were elevated, but lower bounds of the 95% CIs were 0.98 and 0.99.

These studies did not have very good occupational data.

For the radio and telegraph operators studies, the same cohort and occupational determination method was used. The Kliukiene *et al.* (2003) study was identical to the Tynes *et al.* (1996) study, except for a longer follow-up. By the end of May 2002, there were 99 breast cancer cases among the 2619 radio and/or telegraph operators in the cohort. The standardized incidence ratio was 1.30, 95% CI = [1.05 – 1.58].

A nested case-control study was also conducted, using the 99 BC cases and 4 controls per case matched on year of birth ± 5 years for cases born prior to 1920 and ± 1 year for cases born in 1920 or later. It was an update of an earlier study by Tynes *et al.* (1996). The reference category consisted of subjects (all radio and/or telegraph operators) who were not registered in the Norwegian Seamen Registry, i.e., had no history of working on merchant ships. ELF MF exposure was not particularly explicit. It seems to have been assumed that that women who had no history of working on merchant ships had lower MF exposure (ELF and radiofrequency) than those with a history of such work. Spot ELF MF and radiofrequency MF measurements in the radio/telegraph rooms of 2 and 3 ships, respectively, were performed. RF magnetic and electric fields were below the detection level of the instruments at the operator's desks. ELF magnetic fields varied from 0.2 mG to 60 mG at the operator's desks. However, the highest exposures were only to the stretched out leg. "Normal" exposure to the body varied from 1 mG to

2 mG. Thus, exposure was certainly not high.

Tertiles of cumulative exposure at sea were used in the statistical analyses, with adjustment for age-at-first birth and parity. Detailed job histories on each ship were available for each 'exposed' subject. For each ship, the amount of time spent in the radio/telegraph room was estimated by an experienced researcher. A rank of 1-3 was assigned: 1 – 'long voyage' for tankers or dry-cargo ships with longer stays as sea; 2 – 'many calls' for trade ships with several loading and discharge ports; 3 – larger passenger ships. Increasing rank implies increasing percentage of time spent in the radio/telegraph room. Exposure was then calculated by summing the product of the number years of service on ships of each rank by the rank of the ships.

Analyses were conducted for total exposure, and for total exposure with lag times of 10 and 20 years prior to BC diagnosis. Analyses were conducted for (1) all cases and controls, for cases and controls below age 50 in the reference year, and for cases and controls at least age 50 in the reference year, and (2) ER+ and ER- cases.

No OR was statistically significant for any analysis without consideration of ER status. However, there was a statistically significant increasing trend in the ORs over cumulative exposure categories in the analyses for all cases, cases younger than 50, and cases at least age 50. There was also a significant upward trend for a 10 year lag time using all cases. The ORs for the highest exposure category were all elevated, but not significant perhaps because of the sample size.

For analyses by ER status, the only significant finding was for ER- cases, age 50+ in the highest exposure category. There were elevated ORs for all exposure categories for all ER- cases, and for the highest exposure category for ER+ cases and for ER+ cases below age 50.

The authors concluded that "occupational exposure to electromagnetic fields increases the risk of (female) breast cancer" (Kliukiene *et al.*, 2003).

- Loomis *et al.* (1994) investigated BC mortality among female electrical utility workers. This study used U.S. national death certificate information, 1985-1989, to identify cases and controls (without leukemia or brain cancer as a cause or contributing cause of death) and occupations. There were 27,814 women with breast cancer and sufficient occupational information, of whom 68 had an "electrical" occupation. There were 110,750 controls, of whom 199 had an "electrical" occupation. The primary factor limiting the sample size was the availability of occupational information. It should be noted that use of occupational data from death certificates is far from optimal. Statistical adjustments were made for age, ethnicity, and social class. Loomis *et al.* found an elevated risk associated with having an electrical occupation recorded on the death certificate: OR=1.38 ($p<0.05$). The only specific occupation with a statistically significant elevated risk was telephone installers, repairers and line workers: OR=2.17. Electrical engineers and electrical technicians had 'elevated', but not significant risk estimates (OR=1.73 and 1.28). On the other hand, air traffic controllers, telephone operators, data keyers, computer operators, computer programmers did not have 'elevated' risk estimates.

In a letter commenting on the Loomis *et al.* paper, Kantor *et al.* (1995) analyzed essentially the same data set, with the inclusion of data from 1984. They used an industrial hygienist to estimate the probability of occupational ELF MF exposure or video display terminals (0, low, medium or high) among white and black women. The ORs were statistically significant (but not particularly high) for medium or high probability of exposure for both white and black women. When the hygienist actually categorized the level of ELF MF exposure, only medium exposure was associated with a statistically significant OR. High exposure had somewhat lower ORs.

- Forssén *et al.* (2005) published a case-control study of occupational ELF MF exposure and breast cancer. This study may be considered influential, unless reviewed in detail. So considerable detail is provided.

  The Forssén *et al.* (2005) study found no association between occupational ELF MF exposure, as determined by Forssén *et al.* (2005) , and breast cancer. The study is singled out because (1) it is essentially well designed, and (2) has a completely inappropriate ELF MF occupational classification scheme based on either non-representative workers in specific occupations or what should be considered quite suspect individual measurements (Forssén *et al.*, 2004). Many occupational groups which are generally considered to contain higher ELF MF exposed occupations have been classified as low or medium-low exposure.

  ** Forssén *et al.* (2005) did find that seamstresses had statistically significantly elevated risk of breast cancer. However, they classified seamstresses as having medium-low ELF MF exposure. **

Forssén *et al.* (2005) used newly collected exposure data for occupations in which women commonly work (Forssén *et al.*, 2004). The exposure study assessed occupations identified within the Swedish 1980 census. Forty-nine (49) specific occupational titles were identified. Volunteers working in each of these occupations were then ascertained by methods which are not specified. Personal 24-hour ELF MF measurements were obtained on what was presumably supposed to be a typical 24-hour day, using a dosimeter worn at the waist. The volunteers kept a diary so that time periods at work, at home, and elsewhere could be identified. The number of subjects with measurements by occupation ranged from 5 to 24. The total number of subjects measured was 471. There were only 5 observations for Seamstresses, and 5 Radio and Television Assemblers and Repairwomen. The workday measurements were used for classification purposes. In the epidemiologic study of breast cancer, 4 categories of exposure were used: Low (< 1 mG); Medium-Low (1-1.9 mG); Medium-High (2-2.9 mG); and High (≥ 3 mG). The occupations in the categories above 'low' are provided in Table 9. The arithmetic rate of change measure was also calculated. Seamstresses and Radio and Television Assemblers and Repairwomen were both classified as medium-low exposed occupations. The 5 seamstresses measured for exposure had their own small businesses and did not work in apparel manufacturing. They evidently also did not do much sewing. They spent 55% of their workday in fields below 1 mG and only 15% in fields above 3mG. This is only an average of 1 hour and 12 minutes of 'high' exposure during a working day. In the two counties in Sweden in which both the measurement study and the breast cancer case-control study were performed, there was almost no apparel manufacturing (Forssén *et al.*, 2004; personal communication, M. Feychting, 2007). Still, it is difficult to imagine such low exposures among women who actually work as seamstresses.

The cases and controls were obtained from all women who were employed at any time between 1976 and 1999, based on any of the censuses between 1960 and 1990, in either Stockholm or Gotland counties, Sweden. Subjects entered the study in either 1976 or their 15th birthday, which ever came first, and were followed through 1999 or to the date of their initial breast cancer diagnosis. Cases were identified through the Regional Cancer Registry in Stockholm. The referent year was the year of the case's diagnosis. Controls were selected randomly by age and calendar year, apparently matched to cases. Cases could not also be controls. Both cases and controls had to be living in Stockholm or Gotland counties during the referent year. All information, including occupational history, was obtained from registries. 20,400 cases and 116,227 controls were enrolled in the study. Varying numbers of cases and controls were used in the analyses, depending on the availability of occupational and other data. Statistical adjustment was made for age, referent year, parity, and socioeconomic status.

For statistical analyses, exposure was assessed in various ways: (1) ELF MF exposure for the occupation closest to the time prior to the referent year; (2) ELF MF exposure at the most recent census which was at least10 years prior to the referent date; (3) ELF MF exposure at the most recent census when the subject was at least age 35. Analyses were also carried out by (4) splitting the study period at 1985, by (5) only using subjects who either always had low exposure or ever having had high exposure, and by (6) defining low exposure as a median less than 1 mG and a third quartile of less than 1.7 mG and high exposure as a median greater than 2.5 mG and a first quartile including 1.7 mG. With these definitions, high exposed occupations were cashiers, working proprietors in retail trade, air stewardesses, dental nurses, cooks, post office clerks, and kitchen maids. No time latency period was used in the analyses related to (3).

There were no significant or elevated adjusted ORs for analysis (1) using the 4 categories of exposure, either for all BC cases, ER positive cases, or ER negative cases, for age below or at least 50. The referent group had ELF MF exposure below 1 mG. There were no significant or elevated adjusted ORs for analysis (1) using low versus high (separated) exposure categories defined by (6), above.

Finally, in a series of analyses based on exposure 10+ years before the referent year, before age 35 for post-menopausal women, referent year before or after 1985, maximum point exposure, rate of change, and proportion of time exposure was above 3 mG, only a single adjusted OR was significant. The significant OR=0.87 and was for medium-high ELF MF exposure among post-menopausal women before age 35.

It is thus fair to say that Forssén *et al.* (2005) found no relationship between their assessment of ELF MF exposure and breast cancer. The authors do recognize that "(t)he major concern in the study is exposure misclassification".

Their job exposure classification is at odds with other classifications. Forssén *et al.* (2004, 2005) have classified Dental Nurses, Cashiers in Retail Stores and Restaurants, Working Proprietors in Retail Trade, Cooks, and Air Stewardesses as high ELF MF exposure occupations. None of these occupations would be classified as having high ELF MF exposure in any other classification scheme. The common cut-point for high exposure is 10 mG. Cashiers, cooks, and air stewardesses may at times have medium or high exposure, depending on (1) the exposure from scanners, (2) the exposure from microwave ovens, mixers, other motorized kitchen equipment, and (3) the exposure time from sitting near electrical panels on takeoff and landing and in the airplane's kitchen areas.

> ** Forssén *et al.* should conduct a sub-study to determine the actual environment in which the seamstresses in their study worked, the type of machines used (industrial, home; AC or DC operation), and the percent of time spent actually sewing. They also should conduct a study of seamstresses in general in Stockholm and Gotland counties and the in-migration rates. Also, the authors note an occupational category labeled 'textile occupations', which certainly includes seamstresses, but is otherwise undefined in the paper. Textile occupations need to be specified and studied individually, as was done by Hansen *et al.*, 2000. It is important to determine whether the "seamstresses" in the Forssén *et al.* (2005) study have fundamentally different levels of exposure than seamstresses in other studies.**

The only significant occupational finding in this study related to seamstresses. Two analyses were conducted related to seamstresses (Table 10), probably because their exposure assessment was so at odds with every other series of exposure measurements of seamstresses. First, the OR for 'textile occupations', undefined in the paper, versus low ELF MF exposed occupations was 1.37, 95% CI = [1.11 – 1.68]. Second, the OR for 'textile occupations' versus all other occupations, regardless of ELF MF exposure assessment, was 1.33, 95% CI = [1.10 – 1.62]. The authors state that their results "suggest that the increased risk for breast cancer in these occupations might be related to some exposure other than magnetic fields".

'Textile occupations' were not defined, but could certainly have included a multitude of occupations with quite varying chemical exposures, and generally medium or high ELF MF exposures. However, none of the 49 occupational categories, other than seamstress, used in the study appear to relate to textile occupations, if sales and administration are excluded.

The numbers of seamstresses as cases or controls in the study are not provided. However, in the AD studies by Sobel and Davanipour (1995, 1996, 2007), approximately 2% of the controls were seamstresses. Thus, there may have been at least 2000 seamstresses among the controls. Assuming that most, if not all women in "textile occupations" were seamstresses, and based on the OR of "textile occupations" vs ELF MF exposure below 1 mG, the number of seamstresses with BC in the study can be estimated as approximately 475. Rough calculations indicate that if seamstresses are reclassified as having high ELF MF exposure (> 3 mG), the adjusted OR for high occupational ELF MF versus low occupational ELF MF exposure would be about 1.10 and statistically significant. It is worth repeating that the Forssén *et al.* (2004) occupational classification for high ELF MF exposure is (1) not as high as usual and (2) measured workday exposures are unusual for such occupations.

- Forssén *et al.* (2000) conducted an earlier case-control study of occupational and residential ELF MF exposure and breast cancer. The cohort from which the study population was obtained consisted of all Swedish residents who lived within 300 meters of a (high power, 220 or 400 kilovolt) transmission line for at least one year between 1960 and 1985 and were at least age 16 sometime in the period. Subjects in this group living further away from transmission lines essentially had no exposure from such lines. Cases were identified through cancer registries. Controls were randomly selected and matched by age group, residence in the same parish at the time of diagnosis of the case and in the same type of house (single-family/apartment further than 300 meters from the same power line. (The parish/power line criteria were relaxed for 95 cases; a control could not be found for 7 cases.) Residential exposure was calculated from the ELF MF generated by power lines. Occupation information was obtained from census data. An older job- exposure matrix was used to assess occupational ELF MF exposure. Low (< 1.2 mG), medium (1.2 – 1.9 mG), and high (≥ 2.0 mG) exposure categories were selected, based on quartiles. Exposure greater or equal to 2.5 mG was also considered.

  Statistical adjustments were made for the matching variables. Only occupational exposure immediately prior to the diagnosis of BC and only residential exposure at the time of diagnosis was used in the analyses. No information concerning occupations of the subjects was provided. It is unlikely that seamstresses were included in the analyses.

  No significant findings were identified.

  Of 1767 cases and 1766 controls, only 711 and 709, respectively, had residential exposure information, only 744 and 764 had occupational exposure information, and only 197 and 200 had both types of exposure information. For the actual analyses of occupational exposures, with matching variable adjustment, there was complete information for only 440 cases and 439 controls. For analyses using both occupation and residential exposures, and matching variables, there was complete information for only 87 cases and 83 controls.

Partially Positive/Partially Negative Studies

- Coogan et al. (1996, 1998) and McElroy et al. (2007) conducted case-control studies using the same ELF MF exposure classification scheme.
  - The 1996 Coogan et al. study selected breast cancer cases, aged 74 or younger, from the Maine, Wisconsin, Massachusetts, and New Hampshire cancer registries who were diagnosed between April 1988 and December 1991. Controls, aged below 65, were selected from state driver's license lists and were frequency matched to cases by 5-year age intervals. Cases aged below 65 had to have driver's licenses. Controls, aged 65-74, were selected from the Health Care Financing Administration's Medicare beneficiary lists. "Most representative" occupation was obtained via telephone interviews. Occupation duties and industry were obtained if "the occupation was not clear".

    Occupations were coded according to the 1980 Bureau of the Census 3-digit occupational classification. The ELF MF exposure classification scheme identified each of the 3-digit occupation classes as low, medium or high or background (non-exposed) exposure "potential". It is our opinion that the classification scheme is rather deficient: for example,

1. Welders are classified as having **medium** ELF MF potential exposure;
2. Dressmakers (e.g., seamstress) and tailors are classified as having **low** potential for ELF MF exposure;
3. Shoe repairers are classified as having **low** potential for ELF MF exposure;
4. Electrical/Electronic Engineers are classified as having **high** potential for ELF MF exposure;
5. Statisticians and Scientists are classified as having **medium** potential for ELF MF exposure.

In most classification schemes, including that of Sobel-Davanipour et al., welders, dressmakers (seamstresses) are classified as high ELF MF exposed occupations, shoe repairers, electrical/electronic engineers would be classified as medium exposed occupations, and statisticians and scientists would be classified as low exposed occupations.

Nevertheless, the adjusted OR for breast cancer among subjects having occupations with high potential ELF MF exposure versus background was 1.43, with a 95% CI of (0.99 , 2.09). Among pre-menopausal cases with high exposure potential occupations, the adjusted OR was 1.98, with a 95% CI of (1.04, 3.78).

- Coogan and Aschengrau (1998) essentially replicated the earlier Coogan et al. (1996) study, except for adding non-occupational exposure, e.g., homes close to transmission lines, electric heating, bed-warming device. Cases and controls were obtained from Cape Cod, where elevated rates of breast cancer had been observed. Complete work histories (beginning at age 18) were obtained by interview. Jobs were classified using the methodology in Coogan et al. (1996). There were 259 cases and 738 controls. The crude and adjusted ORs were all below 2.0, except for having a "high" ELF MF job at some point and "other ELF MF exposure". The adjusted OR in this case was 2.3. None of the OR estimates was significant.

- McElroy et al. (2007) replicated the initial Coogan et al. (1996) study with female breast cancer subjects obtained from the Massachusetts, New Hampshire, and Wisconsin cancer registries after the close of recruitment for the Coogan et al. (1996, 1998) studies. Occupational ELF MF exposure using the same methodology as in the Coogan et al. (1996, 1998) studies was estimated for each subject's primary occupation. This was a large study: 6213 cases and 7390 controls. None of the adjusted (or unadjusted) ORs were anywhere near statistical significance. (The largest adjusted OR was 1.21.) However, the trend for increasing adjusted (or unadjusted) ORs for all women and for women who were post-menopausal at diagnosis were statistically significant, with p-values between 0.02 and 0.04.

We emphasize that the ELF MF exposure categories are quite inappropriate.

- Peplonska et al. (2007) conducted a case-control study of 2386 incident BC cases (diagnosed in 2000-2003) and 2502 controls. Lifetime occupational histories and known BC risk factors information were obtained. Occupational information included job title, start and stop dates, work activities and duties, and product(s) made and/or service provided. Occupations were coded to the Standard Industrial Classification Manual (1987) and the Standard Occupational Classification Manual (1980). Occupations were characterized as 'white collar' and 'blue collar'. Analyses are provided by occupation and duration, and by industry and duration. Thus, it is generally not possible to identify subjects with significant ELF MF exposure. For example, the following occupations are combined:
    - ✓ electrical, electronic, agricultural, industrial, mechanical, computer, and other engineers;
    - ✓ engineering and related technologists and technicians;
    - ✓ typists, secretaries, stenographers;
    - ✓ hairdressers and cosmetologists;
    - ✓ machine operators and tenders;
    - ✓ printing machine operators and tenders;
    - ✓ textile apparel and furnishing machine operators and tenders;
    - ✓ textile sewing machine operators and tenders;
    - ✓ welders and solderers.

  Analyses by at least somewhat relevant occupational categories for any duration of work are as follows:
    1. Engineers (electrical, electronic, agricultural, industrial, mechanical, computer, and others): OR=2.0, 95% CI = (1.05 , 3.8);
    2. Health record technologists and technicians: OR=2.4; 95% CI = (1.04 , 5.7);
    3. Machine operators and tenders: OR=1.2 95% CI = (1.03 , 1.5);
    4. Printing machine operators and tenders: OR=3.1; 95% CI = (1.4 , 7.0);
    5. Textile apparel and furnishing machine operators and tenders: OR=1.3; 95% CI = (1.03 , 1.5);
    6. Textile sewing machine operators and tenders (a subset of the previous job category): OR=1.2; 95% CI = (0.9 , 1.5);
    7. Welders and solderers: OR=1.2; 95% CI = (0.6 , 2.8).

  None of these seven occupations showed any trend towards increasing risk with duration of work: ≤ 10 years vs > 10 years.

  The analyses by industry are particularly inappropriate.

  The authors used a job exposure matrix (JEM) developed by the National Cancer Institute for a brain cancer study (unreferenced) to evaluate ELF MF exposure and the risk of BC. They identified a statistically significant trend with ORs equal to 1.2, 1.2, and 1.5 for low, medium, high ELF MF exposure. (The actual data were not provided in the paper or online supplementary materials. The authors state that the "excesses in the highest exposure category" were almost completely due to textile apparel and furnishing machine operators and tenders. These employees evidently formed "99%" of the entire high ELF MF exposure group.

With respect to considering ELF MF as a risk factor for breast cancer, the authors would have been better served to use the actual job title and descriptions to form categories of ELF MF exposure. Nevertheless, the authors state that "occupations with potential exposure to magnetic fields deserve further evaluation".

- Ray et al. (2007) conducted a large and potentially valuable study of breast cancer among female textile workers in Shanghai, China. The authors took advantage of a randomized trial of breast self-examination efficacy to conduct a case-cohort study of occupational exposures and BC risk. 1709 BC cases and an age-stratified reference sub-cohort of 3155 non-cases were studied. Hazard ratios were estimated for duration in various job categories and exposure duration by Cox proportional hazards methodology.

  A job exposure matrix was developed for ELF MF exposure (Wernil et al., 2006). Admittedly based on a small number of subjects, the proportion of specific processes in the following textile industry areas were found to result in ELF MF exposure: spinning (75%, 8 of 12); weaving (88.9%, 8 of 9); cutting and sewing (60%, 3 of 5); and maintenance (30%, 3 of 10). There was no information about the extent (in instantaneous or cumulative mG) of the exposure.

  Among the weavers, cutters/sewers, and maintenance female personnel, only cutters/sewers and maintenance personnel with 10 – 20 years of experience had hazard ratios exceeding 1.0: HR=1.61, 95% CI = (1.16 , 2.25) and HR=1.83, 95% CI = (1.01 , 3.32), respectively. There were no indications of any trend. (Note: individual simple calculations of odds ratios for having worked primarily as a weaver, as a cutter/sewer, or as a maintenance person showed no increase or decrease in risk of BC.

  Evidently, no information as to what the ELF MF exposures were for various jobs, e.g., sewer, was collected.

F. Residential Case-Control Studies of ELF MF Exposure as a Risk Factor for Breast Cancer

Residential ELF MF exposure studies and BC have either used wire configuration coding, proximity to high voltage lines, various protocols of room measurements, or a combination of these methods. These studies have generally not found any increased risk of breast cancer (e.g., Feychting *et al.*, 1998; Davis *et al.*, 2002; London *et al.*, 2003; Schoenfeld *et al.*, 2003). Residential studies have measured actual magnetic fields only in current homes of cases and controls, thus homes which might be etiologically relevant are often or usually without actual measurements. Wire configurations and proximity to high voltage lines were at times used for surrogate measures of exposure related to previous homes. Each of these three methods of assessment of the level of exposure leads to significant classification errors. In addition, residential exposures are, almost always, surely relatively low. Individualized exposure, due for example to home sewing, sitting or sleeping near a panel of circuit breakers, sitting near a water pipe (e.g., in the floor or ceiling), is not identified. For homes near high voltage lines, rooms can have dramatically different ambient levels of ELF MF. For these reasons, these studies are not relevant to the purposes of this review.

G. Radiofrequency Exposure and Breast Cancer

There are no epidemiologic studies of radiofrequency MF exposure and breast cancer which do not include ELF MF exposure and which have reasonable data on RF exposure, e.g., Kliukiene *et al.* (2003).

V. SEAMSTRESSES

Conclusion: Seamstresses are, in fact, one of the most highly ELF MF exposed occupations, with exposure levels generally above 10 mG over a significant proportion of the workday. They have also been consistently found to be at higher risk of Alzheimer's disease and (female) breast cancer. This occupation deserves specific attention in future studies.

A. Sobel-Davanipour et al. Studies

Seamstress was the primary occupation among women with high ELF MF exposure in the Sobel *et al.* (1995, 1996b) and Davanipour *et al.* (2007) studies related to AD. No other published AD study has evidently involved populations in which sewing was a somewhat common occupation. In the 5 independent case-control studies presented in the 3 Sobel & Davanipour papers, most of the high ELF MF exposed women (cases and controls) were seamstresses. (Among women in these case- control studies, the Mantel-Haenszel AD odds ratio for seamstresses is 3.13, $p < 0.01$). Information about sewing as a hobby, which at least used to be common, was unavailable. Seamstresses have been shown to have very high ELF MF exposures (e.g., Szabó *et al.*, 2006; Kelsey *et al.*, 2003; Deadman and Infante-Rivard, 2002; Hansen *et al.*, 2000). Forssén *et al.* (2004) measured 5 "seamstresses" who owned independent small businesses and found what they classified as medium-low exposure – a mean of 1.7 mG. These 5 individuals used home sewing machines and evidently did not sew very often. Peplonska *et al.* (2007), using a NCI occupational ELF MF classification scheme found that, at least among women, nearly all high exposures occurred among textile machine operators and tenders. Both Forssén *et al.* (2005) and Peplonska *et al.* (2007) found statistically significantly elevated ORs for breast cancer among seamstresses/textile machine operators and tenders.

Sobel and Davanipour (1996c) measured ELF MF exposure from several home sewing machine models, both AC and DC models, to several parts of the body. The results are provided in Table 10. These results show that (1) high ELF MF exposure occurs to many parts of the body, (2) exposures vary by manufacturer, model, and even by machines of the same model, and (3) exposures depend on whether the machine operates by AC or DC current. For Alzheimer's disease and for breast cancer, it is not known where exposures may be most important. The peripheral Abeta hypothesis, if correct, would indicate that exposure to any location is important for AD. To affect pineal production of melatonin, it is not known whether exposure to the pineal gland is what is most important. For example, a majority of breast cancers causally lower pineal melatonin production. Because the melatonin production rebounds after excision of the tumor, the tumor itself must be secreting something that leads to the decline in melatonin production. Thus, it is conceivable that ELF MF exposure may, at least in some individuals, also lead to the peripheral production of something that also causes a lowering of melatonin production. It is also not known whether ELF MF exposure directly to the breast is etiologically important. Note that the right breast receives higher ELF MF exposure from home sewing machines. No studies of right versus left breast cancer and use of home sewing machines have been published.

B. Examples of Studies with 'Questionable' Seamstress Exposure Assessment:

Swedish and German Studies

Most of the Swedish studies on ELF MF and Alzheimer's disease/dementia or breast cancer (e.g., Forssén et al., 2000, 2004, 2005), Andel et al., 2010, Seidler et al., 2007, Feychting et al., 1998a) have relied on an occupational exposure assessment for seamstresses which significantly under-estimates exposure. For example:

- Seidler et al. (2007) uses governmental census categories which lumps seamstresses together with spinners, weavers, knitters, and dyers, all of whom probably have relatively low exposure. Maximum exposure in this occupational category is given as only1.5 mG, which is below the background levels for seamstresses working in factories.
- Forssén et al. (2004) created a job-exposure matrix for occupational ELF MF exposure among women working in the 49 most common or suspected high ELF MF ISCO job categories in Stockholm County using the Swedish 1980 census (Table 14). (ISCO stands for International Standard Classification of Occupations.) Five (5) to 24 subjects were selected in each of these occupations. Each or many of the ISCO job categories include several different occupations. Thus, workers from subgroups were selected. Sampled workers were instructed to wear their dosimeters for 24 hours and to make diary entries if they need to take off the dosimeter. Seamstresses are described as being rather uncommon in Stockholm County, except possibly for repair of clothing. This may account for the very low ELF MF exposure identified. Seamstresses are listed as having a geometric mean occupational exposure of only 1.7 mG. Only about 15% of their time was about 3 mG exposure. Cooks, kitchen maids, air stewardesses, hairdressers/beauticians all are listed as having greater exposure. Housekeeping service work had comparable exposure levels to seamstresses. As discussed in this report, the research by Davanipour, Sobel, and colleagues demonstrates that actual professional seamstresses have a very different exposure experience.

A re-analysis of the data in these studies with the job exposure classification scheme in the Davanipour & Sobel studies (Table 11) would be useful.

Note: The Kliukiene et al. study (2004) from Norway used a rather unique four division scale depending on how many hours of occupational exposure were above 1 mG per week and is thus not related to this discussion.]

Note: Qiu et al., 2004 exposure assessment problems has been discussed in Section D.3.4, above.

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Figure 1: Hypothesized Biological Pathway from ELF MF Exposure to AD Development (from Sobel & Davanipour, 1996a)

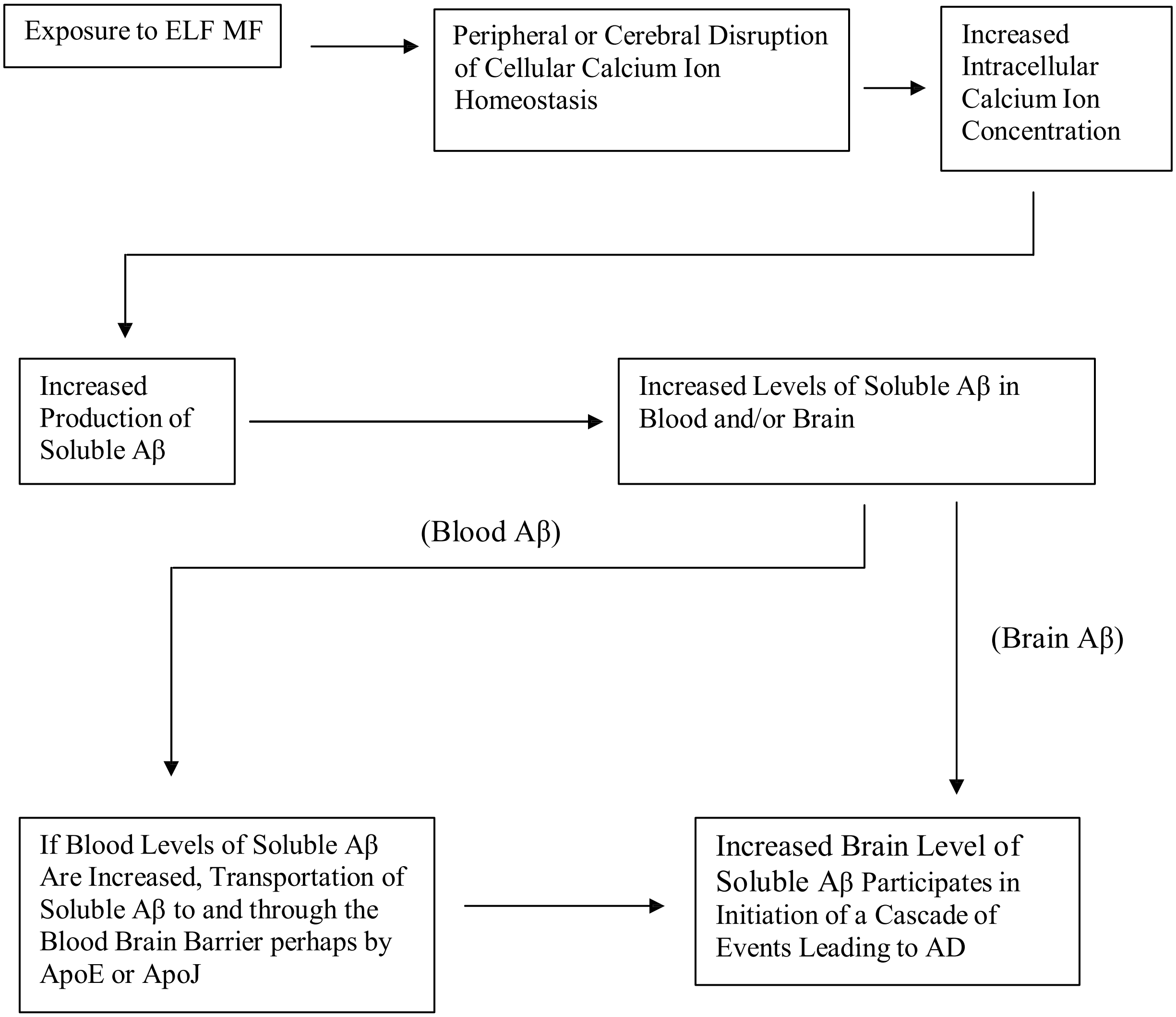
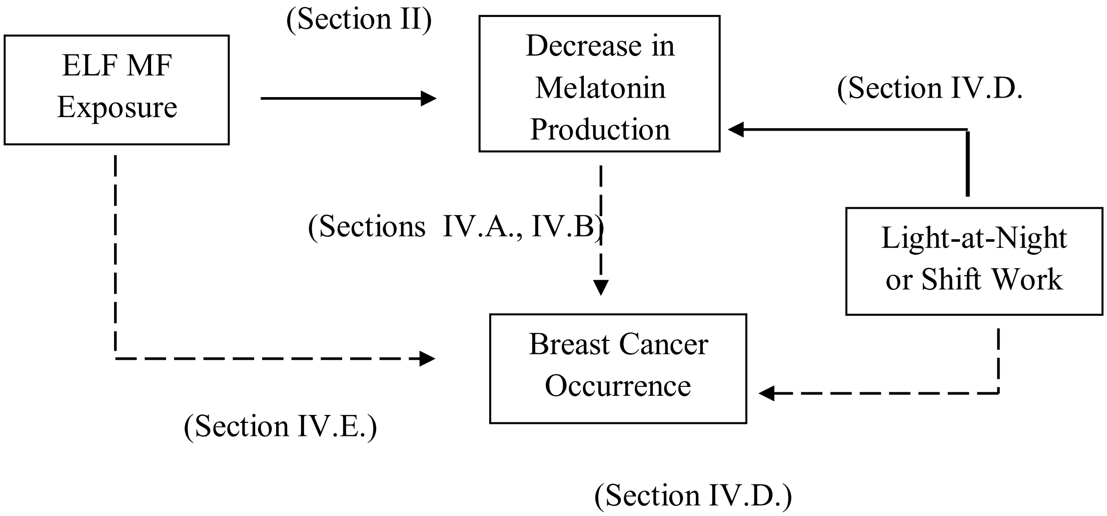

Exposure to ELF MF
Peripheral or Cerebral Disruption of Cellular Calcium Ion Homeostasis
Increased Intracellular Calcium Ion Concentration
Increased Production of Soluble Aβ
Increased Levels of Soluble Aβ in Blood and/or Brain
(Blood Aβ)
(Brain Aβ)
If Blood Levels of Soluble Aβ Are Increased, Transportation of Soluble Aβ to and through the Blood Brain Barrier perhaps by ApoE or ApoJ
Increased Brain Level of Soluble Aβ Participates in Initiation of a Cascade of Events Leading to AD

Figure 2: Outline of the Evidence that ELF MF Exposure Causes Breast Cancer through Decreases in Melatonin Production – with Section References


(Section II)
ELF MF Exposure
Decrease in Melatonin Production
(Section IV.D.
(Sections IV.A., IV.B)
Light-at-Night or Shift Work
Breast Cancer Occurrence
(Section IV.E.)
(Section IV.D.)

Note: Dashed lines indicate studies directly relating ELF MF exposure, light-at-night, or shift work to breast cancer occurrence.

Table 1: Baseline Data Results from the 1999 Mayeux *et al*. Paper: Means (Standard Deviation)

Variable	Cognitively Normal at Follow-Up	Developed AD (3.6 Year Average Follow-Up)
Sample Size (n)	105	64
Age	73.4 (5.3)	77.4 (5.9)[a]
Education	9.3 (4.6)	7.5 (3.8)[a]
$A\beta_{1\text{-}40}$ (pg/ml)	111.8 (44.1)	134.7 (46.4)[a]
$A\beta_{1\text{-}42}$ (pg/ml0	51.5 (42.0)	82.4 (68.8)[a]
$A\beta_{1\text{-}42}$/ $A\beta_{1\text{-}42}$	0.51 (0.41)	0.67 (0.56)[b]

Notes: Cognitively normal was determined at baseline by the global Cognitive Dementia Rating (CDR) scale with CDR=0 being normal. AD was diagnosed based on a CDR of 0.5 or 1.0, and clinical, functional and neuropsychological assessment as specified by the NINCDS-ADRDA criteria. [a] $p \leq 0.0001$; [b] $p < 0.05$.

Table 2: Baseline Data Results from the 2003 Mayeux *et al*. Paper: Means (Standard Deviation)

Variable	Cognitively Normal At Follow-Up	Developed AD (Up to 10 Year Follow-Up)
Sample Size (n)	365	86
Age	75.5 (5.9)	79.3 (6.6)[a]
Education	9.0 (4.6)	6.8 (4.5)[a]
$A\beta_{1\text{-}40}$ (pg/ml)	133.3 (61.9)	136.2 (46.7)[c]
$A\beta_{1\text{-}42}$ (pg/ml)	58.8 (32.9)	76.5 (59.8)[b]
$A\beta_{1\text{-}42}$/ $A\beta_{1\text{-}42}$	0.48 (0.3)	0.61 (0.53)[b]

Notes: Cognitively normal was determined at baseline by the global Cognitive Dementia Rating (CDR) scale with CDR=0 being normal. AD was diagnosed based on a CDR of 0.5 or 1.0, and clinical, functional and neuropsychological assessment as specified by the NINCDS-ADRDA criteria. [a] $p \leq 0.001$; [b] $p < 0.05$; [c] Not Significant.

Table 3: Post-Work Levels of Aβ1-40, Aβ1-42, Aβ1-42/Aβ1-42 by ELF MF exposure among Electrical Workers in the Noonan *et al*. (2002a) Study

ELF MF Exposure	$A\beta_{1-40}$ (pg/ml)	$A\beta_{1-42}$ (pg/ml)	$A\beta_{1-42}/A\beta_{1-42}$	Sample Size
< 0.5 mG	125	136	1.03	20
0.5 – 0.99 mG	137	163	1.11	25
1.0 – 1.99 mG	128	166	1.19	8
≥ 2.0 mG	156	262	1.46	7

Table 4: Correlation (Corr) between Post-Work Creatinine-Adjusted aMT6s and Amyloid Beta by Number of Minutes between Samples in the Noonan *et al.* (2002a) Study

Number of Minutes	Sample Size	$A\beta_{1\text{-}42}$		$A\beta_{1\text{-}40}$		$A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$	
		Corr	p-Value	Corr	p-Value	Corr	p-Value
All Subjects	60	-0.25	0.057	-0.19	0.144	-0.23	0.080
≤ 90	46	-0.30	0.047	-0.22	0.154	-0.27	0.080
≤ 60	37	-0.37	0.027	-0.25	0.150	-0.37	0.029
≤ 30	23	-0.43	0.054	-0.28	0.224	-0.42	0.059

Table 5: Amyloid Beta Levels by Tertile of Post-Shift Creatinine-Adjusted aMT6s Levels in the Noonan *et al*. (2002a) Study

aMT6s/Cr Tertiles* (ng/mg)	$A\beta_{1\text{-}42}$ Mean**	$A\beta_{1\text{-}42}$ 95% CI	$A\beta_{1\text{-}40}$ Mean**	$A\beta_{1\text{-}40}$ 95% CI	$A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ Mean**	$A\beta_{1\text{-}42}/A\beta_{1\text{-}40}$ 95% CI
≤ 1.38	177	[112–258]	133	[111–156]	1.30	[0.86–1.74]
1.39–3.3	214	[120–334]	147	[125–170]	1.33	[0.85–1.90]
> 3.3	123	[58–180]	123	[108–139]	0.82	[0.49–1.26]

* n=60 subjects in each tertile
** geometric mean averaged over the work shift

Table 6: Percentages of Subjects with Medium to High ELF MF Occupations Exposure

STUDY	CASES	CONTROLS
Sobel *et al.* (1995a)	9.3 %	3.4 %
Sobel *et al.* (1996b)	12.0 %	5.3 %
Davanipour *et al.* (2007)	7.4 %	3.8 %
Harmanci *et al.* (2003)	10.5 %	3.1 %
Feychting *et al.* (1998a)	43.0 %	23.0 % & 19.0 %[#]
Graves *et al.* (1999)	19.1 % & 21.4 %	21.4 % & 22.5 %[^]
Qiu *et al.* (2004)	28.2 %[*]	28.8 %[*]
	34.2 %[**]	42.7 %[**]
	Cases & Controls Combined	
Feychting *et al.* (1998)	11.1 %	
Håkansson *et al.* (2003)	80.5 % - likely exposed engineering industry workers	
Johansen *et al.* (2000)	56 % - electrical company workers	
Savitz *et al.* (1998a)	electric utility cohort – percentage not supplied	
Savitz *et al.* (1998b)	23.9 %	

# Two control groups;
^ Two industrial hygienists
* Based on estimated daily exposure in principal occupation;
** Based on estimated daily exposure in all occupations

Note: The Huss et al. (2009) study was longitudinal and the abstract for the Chang et al. (2004) study did not provide the percentages of cases or controls with high ELF MF exposure.

Table 7: **Odds Ratios for the ELF MF and AD Studies***

Study	Risk Estimate (OR)	95% CI	p-value
Sobel *et al.* (1995) (late-onset; L vs M/H)			
	3.0	1.6 – 5.4	< 0.001
Sobel *et al.* (1996b) (late-onset; L vs M/H)			
	3.9	1.5 – 10.6	0.006
Feychting *et al.* (1998) (mostly late-onset; last occupation; by control group)			
(exposure ≥ 2 mG)	2.4	0.8 – 6.9	--**
	2.7	0.9 – 7.8	--**
(exposure ≥ 5 mG)	4.1	0.7 – 23.5	--**
	8.3	1.1 – 62.7	--**
Graves *et al.* (1999) (late-onset; ever exposed)			
	0.95	0.4 – 2.4	--**
	0.74	0.3 – 2.4	--**
Harmanci *et al.* (2003) (late-onset; exposure as defined in Sobel *et al.* (1995, 1996b)			
	4.0	1.0 – 15.8	--**
Qiu *et al.* (2004) (age ≥ 75; exposure: ≥ 2 mG)			
Men	2.3	1.0 – 5.1	--**
Women	0.8	0.5 – 1.1	--**
Davanipour *et al.* (2007) (exposure as defined in Sobel *et al.* (1995, 1996b)			
M/H vs L	2.2	1.2 – 3.9	< 0.02
H vs L	2.7	0.8 – 9.1	< 0.11
Chang *et al.* (2004) (age: 66-102; exposure: "early exposure to magnetic fields")			
Exp vs No Exp	2.49	0.96 – 6.45	--**

* Studies use various types of controls and definitions of ELF MF exposure. See text.
** p-values were not provided.

Note: the Huss *et al.* (2009) study was longitudinal and is therefore not in this table.

Table 8: Mean ELF MF Exposures (mG) for Home Sewing Machines by Body Location: Continuous 2-Minute Measurements (Sobel & Davanipour, 1996c)

Sewing Machine	Background	Head	Breast		Pelvic Area	Thigh		Knee		Lower Right Arm	Right Hand	Foot Pedal
			Left	Right		Left	Right	Left	Right			
Alternating Current Machines (older machines)												
Bernina 811	0.6	18.6	5.6	12.9	26.9	11.7	90.1	8.9	13.5	251.1	57.0	86.1
Bernina 811	0.9	1.7	2.6	5.4	8.2	4.5	11.6	6.8	36.5	77.1	31.7	102.0
Bernina 817	0.6	8.4	9.6	23.5	41.9	19.1	30.6	9.2	35.4	724.6	135.6	NA
Bernina 817	1.2	12.1	14.2	33.9	51.0	10.3	588.5	8.8	125.7	753.0	132.4	NA
Brother 920D	0.7	2.4	2.1	2.3	1.1	1.3	1.5	1.9	2.3	8.5	16.0	6.2
Necchi Type 525	0.3	5.1	2.0	1.1	2.5	1.1	2.4	2.0	5.1	25.9	22.6	5.9
Sears Kenmore	0.2	1.2	1.9	4.9	5.5	2.2	5.3	2.5	15.8	26.0	17.9	13.8
Singer 625	0.3	4.6	3.6	5.6	5.5	3.9	6.6	6.4	17.2	...	...	...
Singer 5932	0.5	1.2	0.9	2.0	2.7	1.1	2.5	1.0	4.1	8.6	23.0	2.9
Singer 6212C	0.3	7.0	2.8	6.4	2.0	1.4	2.2	1.4	1.9	31.0	26.2	4.4
Viking Husqvarna 6020	0.8	1.5	1.3	1.5	2.7	1.4	2.0	3.1	9.1	5.9	24.9	62.3
White 1410	0.2	2.2	1.6	1.1	1.1	3.2	10.8	4.2	67.5	20.8	18.3	2.8
Direct Current Machines (newer machines)												
Bernina 1000	1.0	1.3	1.6	2.3	2.9	1.9	2.5	2.8	11.2	8.1	41.2	798.0
Bernina 1090S	1.0	1.2	1.6	1.6	1.7	1.2	1.3	1.5	7.7	3.3	22.9	1.0
Elna Diva 900	1.6	5.1	3.9	4.1	4.1	3.0	3.1	3.2	8.4	40.4	57.1	1.8
Singer 3317C	0.7	3.4	1.6	2.9	2.2	2.1	2.2	1.5	11.3	22.1	25.8	5.8
Singer 9015	0.7	2.5	1.9	3.3	4.9	1.7	4.3	2.1	26.2	7.0	28.9	2.3
Viking Husqvarna 500	1.0	3.7	2.7	5.0	3.9	1.8	2.8	2.7	13.8	24.9	39.4	1.1
Percent > 2.0 mG	0%	67%	50%	78%	83%	50%	89%	72%	94%	100%	100%	80%

Note: The Bernina 1000, Bernina 1090S, Elna Diva 900, Singer 3317C, Singer 9015 and Viking Husqvarna 500 were brand new. The Singer 5932, Singer 6212C, and Brother 920D were 3-10 years old. The Bernina 811 and 817 machines, the Sears Kenmore, the Singer 625 the Viking Husqvarna 6020 are probably at least 15 years old. Both the White and the Necchi are fairly old. NA = not applicable, i.e., there was no foot pedal. "..." = no measurements were taken, e.g., because of machine malfunction.

Table 9: **Classification of Occupations in Forssén *et al.* (2005)**

Classification	Occupation	24-Hour Geometric Mean Average (mG)
High (≥ 3 mG)	Dental Nurse	3.0
	Air Stewardesses	3.0
	Cooks	3.1
	Working Proprietors Retail Trade	3.4 in
	Cashiers in Retail Stores and Restaurants	4.5
Medium-High (2 – 2.9 mG)	Computer Operators	2.0
	Motor Vehicle Drivers	2.0
	Shop Managers	2.1
	Shop Assistants	2.1
	Hairdressers/Beauticians	2.1
	Bank Clerks	2.2
	Kitchen Supervisors	2.4
	Post Office Clerks	2.5
	Waitresses in Restaurants and School Kitchens	2.5
	Kitchen Maids	2.8
Medium-Low (1 – 1.9 mG)	Registered Nurses	1.0
	System Analysts/Programmers	1.2
	Telephone Operators	1.5
	Radio & Television Assemblers and Repairwomen	
	Seamstresses	1.6

Table 10: Odds Ratio Estimates for Textile Occupations in the Forssén *et al.* (2005) Study

Comparison	OR	95% Confidence Interval
Textile Occupations vs Occupations with 24-Hour Exposure Below 1 mG	1.37	[1.11 , 1.68]
Textile Occupations vs All Other Occupations (Regardless of ELF MF Exposure)	1.33	[1.10 , 1.62]

Table 11: Sobel-Davanipour Occupations Classified as Being Likely to Have Resulted in Medium or High ELF MF Exposure

Medium Exposure	High Exposure
Beautician	Cutter
Carpenter	Power Plant Operator
Clothes Inspector: Manufacturing Company	Repair Sewing Machines
Electric Lineman	Seamstress/Tailor
Electrician	Welder
Electronics Technician	
Electronic Assembler	
Equipment Repair	
Fabric Cutter	
Foam Cutter	
Forklift Operator	
Furniture Maker	
Machine Operator	
Machinery Repair	
Machinist (	
Newspaper Pressman	
Presser: Clothing Manufacturing Company	
Seamstress/Tailor – Part-Time	
Sheet Metal Machine Operator	
Shoemaker/Shoe Repairer	
Typist	
Upholstery; Re-Upholstery	
Welder - Parttime	
Wood Cutter; Machinery Repair - Forestry	
Wood Sander – Furniture	

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SECTION 14

Evidence for Breast Cancer Promotion

(Melatonin Studies in Cells and Animals)

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Introduction

The subject of breast cancer and studies of melatonin has a long and rich history replete with destroyed scientific reputations and career-ending charges of misconduct of scientists who have contributed stellar scientific work that has proved extremely inconvenient for governmental agencies and military and industrial interests (Liburdy). References are given in each section below to facilitate locating the pertinent references for each section.

II. Melatonin and ELF-EMF

Evidence which supports a possible mechanism for ELF-EMF and breast cancer is the consistent finding (in five separate labs) that environmental levels of ELF-EMF can act at the cellular level to enhance breast cancer proliferation by blocking melatonin's natural oncostatic action in MCF-7 cells (Liburdy, 1993; Luben et al, 1996; Morris et al, 1998; Blackman et al, 2001; Ishido, et al, 2001). ELF-EMF levels between 0.6 and 1.2 μT have been shown to consistently block the protective effects of melatonin.

The series of papers reporting increased breast cancer cell proliferation when ELF-EMF at environmental levels negatively affects the oncostatic actions of melatonin in MCF-7 cells should warrant new public exposure guidelines or planning target limits for the public, and for various susceptible segments of the population.

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III. Tamoxifen and ELF-EMF

Girgert et al (2005) reported that *"the anti-estrogenic activity of tamoxifen is reduced in two subclones of MCF-7 cells under the influence of ELF/EMF to different extent. Dose-response curves of the growth-inhibitory effect of tamoxifen are shifted towards higher concentrations leading to a reduced growth inhibition at a given concentration. Our observations confirm results from a previous report describing a reduced inhibitory effect of tamoxifen at* 1^{-7} *M from 40% to only 17% by exposure to an EMF of 1.2 µT"* (Harland t al, 1997). Further, Girgert et al conclude that "*From a medical point of view, it is disturbing that maximal induction of cell proliferation by tamoxifen at a field strength of 1.2 µT is observed at concentration of* 10^{-6} *M. This is exactly the serum concentration achieved in BC patients under standard oral therapy."* (De Cupis et al, 1997).

The Girgert et al paper confirms prior findings that environmental level ELF-EMF inhibits the antiproliferative action of tamoxifen in MCF-7 human breast cancer cells. Four other papers reporting this effect include Liburdy et al, 1997; Harland et al, 1997; Harland et al, 1999; and Blackman et al, 2001).

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VI. Male Breast Cancer Studies

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VII. Conclusions

Conclusion: The constellation of relevant scientific papers providing mutually-reinforcing evidence for an association between power-frequency electromagnetic fields (ELF-EMF) and breast cancer is strongly supported in the scientific literature.

Conclusion: ELF at environmental levels negatively affects the oncostatic effects of both melatonin and tamoxifen on human breast cancer cells. Numerous epidemiological studies over the last two decades have reported increased risk of male and female breast cancer with exposures to residential and occupational levels of ELF. Animal studies have reported increased mammary tumor size and incidence in association with ELF exposure.

Conclusion: ELF limits for public exposure should be revised to reflect increased risk of breast cancer at environmental levels possibly as low as 2 mG or 3 mG; certainly as low as 4 mG.

SECTION 15

Evidence for Disruption by the Modulating Signal

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Fundamental exposure parameters that must be considered when establishing a mode (or mechanism) of action for non-thermal EMF-induced biological effects.

- A. Intensity
- B. Frequency
- C. Static Magnetic Field
- D. Electric & Magnetic Components
- E. Sine and Pulsed Waves
- F. Mechanisms

IV. Problems with Segregation of Effects by Artificial Frequency Bands that Ignore Modulation

- A. Suggested Research

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I. Introduction

Modulation signals are one important component in the delivery of EMF signals to which cells, tissues, organs and individuals can respond biologically. At the most basic level, modulation can be considered a pattern of pulses or repeating signals which have specific meaning in defining that signal apart from all others. Modulated signals have a specific 'beat' defined by how the signal varies periodically over time. Pulsed signals occur in an on-off pattern, which can either be smooth and rhythmic, or sharply pulsed in quick bursts. Amplitude and frequency modulation involves two very different processes where the high-frequency signal, called the carrier wave, has a low-frequency signal that is superimposed on or 'rides' on the carrier frequency. In amplitude modulation, the lower-frequency signal is embedded on the carrier wave as changes in its amplitude as a function of time, whereas in frequency modulation, the lower-frequency signal is embedded as slight changes in the frequency of the carrier wave. Each type of low-frequency modulation conveys specific 'information', and some modulation patterns are more effective (more bioactive) than others depending on the biological reactivity of the exposed material. This enhanced interaction can be a good thing for therapeutic purposes in medicine, but can be deleterious to health where such signals could stimulate disease-related processes, such as increased cell proliferation in precancerous lesions. Modulation signals may interfere with normal, non-linear biological functions. More recent studies of modulated RF signals report changes in human cognition, reaction time, brainwave activity, sleep disruption and immune function. These studies have tested the RF and ELF-modulated RF signals from emerging wireless technologies (cell phones) that rely on pulse modulated RF to transmit signals. Thus modulation can be considered as information content embedded in the higher frequency carrier wave that may have health consequences beyond any effect from the carrier wave directly.

In mobile telephony, for example, modulation is one of the underlying ways to categorize the radiofrequency signal of one telecom carrier from another (TDMA from CDMA from GSM). Modulation is likely a key factor in determining whether and when biological reactivity might be occurring, for example in the new technologies which make use of modulated signals, some modulation (the packaging for delivery for an EMF 'message') may be bioactive, for example, frequencies are similar to those found in brain wave patterns. If a new technology happens to use brain wave frequencies, the chances are higher that it will have effects, in comparison, for example, to choosing some lower or higher modulation frequency to carry the same EMF information to its target. This chapter will show that other EMF factors may also be involved in determining if a given low-frequency signal directly or as a modulation of a radiofrequency wave can be bioactive. Such is the evolving nature of information about modulation. It argues for great care in defining standards that are intended to be protective of public health and well-being. This section describes some features of exposure and physiological conditions that are required in general for non-thermal effects to be produced, and specifically *to illustrate how modulation is a fundamental factor which should be taken into account in public safety standards*.

II. The Old Standards (Based on Heating and Electric Current Flow in Tissues)

It is universally accepted that radiofrequency radiation (RFR) can cause tissue heating and that extremely low frequency (ELF) fields, e.g., 50 and 60 Hz, can cause electrical current flows that shock and even damage or destroy tissues. These factors alone are the underlying bases for present exposure standards. EMF exposures that cause biological effects at intensities that do not cause obvious thermal changes, that is, effects via non-thermal mechanisms, have been widely reported in the scientific literature over the last several decades. The current public safety limits do not take modulation into account and thus are no longer sufficiently protective of public health where chronic exposure to pulsed or pulse-modulated signal is involved, and where sub-populations of more susceptible individuals may be at risk from such exposures.

III. Laboratory Studies

Published laboratory studies have provided evidence for more than 40 years on bioeffects at much lower intensities than cited in the various widely publicized guidelines for limits to prevent harmful effects. Many of these reports show EMF-caused changes in processes associated with cell growth control, differentiation and proliferation which are biological processes of considerable interest to scientists who study the molecular and cellular basis of cancer. EMF effects have been reported in gene induction, transmembrane signaling cascades, gap junction communication, immune system action, rates of cell transformation, and breast cancer cell growth. These reports have cell growth control as a common theme. Other more recent studies on brainwave activity, cognition and human reaction time lend credence to modulation (pulsed RF and ELF-modulated RF) as a concern for wireless technologies, most prominently from cell phone use.

Experimental results are described below to illustrate the influence of each EMF parameter, while also demonstrating that it is highly unlikely the effects are due to EMF-caused current flow or heating.

Several papers in the 1960s and early 1970s reported that ELF fields could alter circadian rhythms in laboratory animals and humans. In the latter 1960s, a paper reported that the EMF environment in planned space capsules could cause human response time changes, i.e., the interval between a signal and the human response (Hamer, 1968). Subsequent experiments by that research group were conducted with monkeys, and showed similar response time changes and also EEG pattern changes (Gavalas, 1970; Gavalas-Medici, 1976). The investigators shifted the research subject to cats and observed EEG pattern changes, ability to sense and behaviorally respond to the ELF component of RFR, and the ability of minor electric current to stimulate the release of an inhibitory neurotransmitter, GABA, and simultaneous release of a surrogate measure, calcium ions, from the cortex (Kaczmarek, 1973, 1974). At this time the investigators adopted newly hatch chickens as sources of brain tissue and observed changes in the release of calcium ions from in vitro specimens as a function of ELF frequency directly or as amplitude modulation (‘am’) of RFR (RFRam) (Bawin, 1975, 1976, 1978a, 1978b; Sheppard, 1979). Tests of both EMF frequency and intensity dependences demonstrated a single sensitive region (termed 'window') over the range of frequency and intensity examined. This series of papers showed that EMF-induced changes could occur in several species (human, monkey, cat and chicken), that calcium ions could be used as surrogate measures for a neurotransmitter, that ELF fields could produce effects similar to RFRam (note: without the 'am', there was no effect although the RFR intensity was the same), and that the dose and frequency response consisted of a single sensitivity window.

An independent research group published a series of papers replicating and extending this earlier work (Blackman et al., 1979, 1980a, 1980b, 1981, 1982, 1985, 1988a, 1988b, 1989, 1990; Joines and Blackman et al., 1981a, 1981b, 1986). These papers reported multiple windows in intensity and in frequency within which calcium changes were observed in the chick brain experimental systems under EMF exposure. Three other independent groups reported intensity and frequency windows for calcium, neurotransmitter or enolase release under EMF exposure of human and animal nervous system-derived cells in vitro (Dutta et al., 1984, 1989, 1992, 1994), of rat pancreatic tissue slices (Albert et al., 1980), and of frog heart (Schwartz et al., 1990) but not atrial strips in vitro (Schwartz et al., 1993). This series of papers showed that multiple frequency and intensity windows were a common phenomenon that required the development of new theoretical concepts to provide a mechanism of action paradigm.

Additional aspects of the EMF experiments with the chick brain described by Blackman and colleagues, above, also revealed critical co-factors that influenced the action of EMF to cause changes in calcium, including the influence of the local static magnetic field, and the influence of physico-chemical parameters, pH, temperature and ionic strength of the bathing solution surrounding the brain tissue during exposure. This information provides clues for and constraints on any theoretical mechanism that is to be developed to explain the phenomenon. These factors demonstrate that the current risk assessment paradigms, which ignore them, are incomplete and thus may not provide the level of protection currently assumed.

The detailed set of frequency and intensity combinations under which effects were observed, were all obtained from chickens incubated for 21 days in an electrically heated chamber containing 60-Hz fields. Tests were performed to determine if the 60-Hz frequency of ELF fields (10 volts per meter in air) during incubation, i.e., during embryogenesis and organogenesis, would alter the subsequent calcium change responses of the brain tissue to EMF exposure. The published papers (Blackman et al., 1988b; Joines et al., 1986) showed that the brain tissue response was changed when the field during the incubation period was 50 Hz rather than 60 Hz. This result is consistent with an anecdotal report of adult humans, who were institutionalized because of chemical sensitivities, were also responsive to EMF fields that were present in the countries where they were born and raised (Blackman, 2006). This information indicates there may be animal and human exposure situations where EMF imprinting could be an important factor in laboratory and epidemiological situations. EMF imprinting, which may only become manifest when a human is subjected to chemical or biological stresses, could reduce ability to fight disease and toxic insult from environmental pollution, resulting in a population in need of more medical services, with resulting lost days at work.

Fundamental exposure parameters that must be considered when establishing a mode (or mechanism) of action for non-thermal EMF-induced biological effects.

A. Intensity

There are numerous reports of biological effects that show intensity "windows", that is, regions of intensity that cause changes surrounded by higher and lower intensities that show no effects from exposure. One very clear effect is 16-Hz, sine wave-induced changes in calcium efflux from brain tissue in a test tube because it shows two very distinct and clearly separated intensity windows of effects surrounded by regions of intensities that caused no effects (Blackman et al., 1982). There are other reports for similar multiple windows of intensity in the radiofrequency range (Blackman et al., 1989; Dutta et al., 1989, 1992; Schwartz et al., 1990). Note that calcium ions are a secondary signal transduction agent active in many cellular pathways. These results show that intensity windows exist, they display an unusual and unanticipated "non linear" (non-linear and non-monotonic) phenomenon that has been mostly ignored in all risk assessment and standard setting exercises, save the National Council for Radiation Protection and Measurements. (NCRP) 1986 publication. Protection from multiple intensity windows has never been incorporated into any risk assessment; to do so would call for a major change in thinking. These results mean that lower intensity is not necessarily less bioactive, or less harmful.

Multiple intensity windows appeared as an unexpected phenomenon in the late 1970s and 1980s. There has been one limited attempt to model the phenomenon (Thompson et al., 2000). However, there are publications from two independent research groups showing multiple intensity windows for 50 MHz, 147 MHz, and 450 MHz fields when amplitude-modulated at 16 Hz using the calcium ion release endpoint in chicken brains, in vitro. The incident intensities (measured in air) for the windows at the different carrier frequencies do not align at the same values. However, Joines et al., (1981a, 1981b) and Blackman et al. (1981) noted the windows of intensity align across different carrier frequencies if one converts the incident intensity to the intensity expected within the sample at the brain surface, but correcting for the different dielectric constants in the samples at the different carrier frequencies. The uniqueness of this response provides a substantial clue to theoreticians but it is interesting that no publications have appeared attempting to address this relationship. It is obvious that this phenomenon is one that needs further study.

B. Frequency

Frequency-dependent phenomena are common occurrences in nature. For example, the human ear only hears a portion of the sound that is in the environment, typically from 20 to 20000 Hz, which is a frequency "window." Another biological frequency window can be observed for plants grown indoors. Given normal indoor lighting the plants may grow to produce lush vegetation but not produce flowers unless illuminated with a lamp that emits a different spectrum of light. Similarly, there are examples of EMF-caused biological effects that occur as a result of EMF of concern to us in a frequency-dependent manner that cannot be explained by current flow or heating. The examples include reports of calcium ion efflux from brain tissue in vitro at low frequency (Blackman et al., 1988a, 1988b) and at high frequency (Blackman et al., 1981; Joines and Blackman, 1981). The bioactive frequency regions observed in these studies have never been explicitly considered for use in any EMF risk assessments, thus demonstrating the incomplete nature of current exposure limits.

There are also EMF frequency-dependent alterations in the action of nerve growth factor (NGF) to stimulate neurite outgrowth (growth of primitive axons or dendrites) from a peripheral-nerve-derived cell (PC-12) in culture (Blackman et al., 1995, 1999; Trillo et al., 1996). The combined effect of frequency and intensity is also a common occurrence in both the sound and the light examples given above. Too much or too little of either frequency or intensity show either no or undesirable effects. Similarly, in low intensity EMF work, "islands" of effective combinations of intensity and frequency are surrounded by a "sea" of null effects (Blackman et al., 1988a). Although the mechanisms responsible for these effects have not been establish, the effects represent a heretofore unknown phenomenon that may have ramifications for risk assessment and standard setting. Nerve growth and neurotransmitter release that can be altered by different combinations of EMF frequencies and intensities, especially in developing organisms like children, could conceivably produce over time a subsequent altered ability to successfully or fully respond behaviorally to natural stressors in the adult environment; research is urgently need to test this possibility in animal systems.

Nevertheless, this phenomenon is ignored in the development of present exposure standards that rely primarily on biological responses to intensities within a relatively narrow band of frequencies, based on an energy deposition endpoint.

C. Static Magnetic Field

The magnetic field of the earth at any given location has a relatively constant intensity as a function of time. However, the intensity value, and the inclination of the field with respect to the gravity vector, varies considerable over the face of the earth. More locally, these features of the earth's magnetic field can also vary by more than 20% inside man-made structures, particularly those with steel support structures. There are many reports of EMF-caused effects being dependent on the static magnetic field intensity (cf. Blackman et al., 1985) and of its orientation, with respect to an oscillating magnetic field (Blackman et al., 1990; Blackman et al., 1996). One aspect common to many of these reports is that the location in the active frequency band is determined by the intensity of the static magnetic field. There have been many attempts to explain this phenomenon but none has been universally accepted. However, it is clear that if a biological response depends on the static magnetic field intensity, and even its orientation with respect to an oscillating field, then the conditions necessary to reproduce the phenomenon are very specific and might easily escape detection (cf. Blackman and Most, 1993). The consequences of these results are that there may be exposure situations that are truly detrimental (or beneficial) to organisms but that are insufficiently common on a large scale that they would not be observed in epidemiological studies; they need to be studied under controlled laboratory conditions to determine impact on health and wellbeing.

D. Electric & Magnetic Components

Both the electric and the magnetic components have been shown to directly and independently cause biological changes. There is one report that clearly distinguishes the distinct biological responses caused by the electric field and by the magnetic field. Marron et al. (1988) show that electric field exposure can increase the negative surface charge density of an amoeba, Physarum polycephalum, and that magnetic field exposure of the same organism causes changes in the surface of the organism to reduce its hydrophobic character. Other scientists have used concentric growth surfaces of different radii and vertical magnetic fields to determine if the magnetic or the induced electric component is the agent causing biological change. Liburdy (1992), examining calcium influx in lymphocytes, and Greene et al. (1991), monitoring ornithine decarboxylase (ODC) activity in cell culture, showed that the induced electric component was responsible for their results. In contrast, Blackman et al. (1993a, 1993b) monitoring neurite outgrowth from two different clones of PC-12 cells and using the same exposure technique used by Liburdy and by Greene showed the magnetic component was the critical agent in their experiments. EMF-induced changes on the cell surface, where it interacts with its environment, can dramatically alter the homeostatic mechanisms in tissues, whereas changes in ODC activity are associated with the induction of cell proliferation, a desirable outcome if one is concerned about wound healing, but undesirable if the concern is tumor cell growth. This information demonstrates the multiple, different ways that EMF can affect biological systems. Current analyses for risk assessment and standard setting have ignored this information, thus making their conclusions of limited value.

E. Sine and Pulsed Waves

Important characteristics of pulsed waves that influenced the number and characteristics of the sine wave representations include the following: 1) frequency, 2) pulse width, 3) intensity, 4) rise and fall time, and 5) the frequency, if any, within the pulse ON time. Chiabrera et al. (1979) showed that pulsed fields caused de-differentiation of amphibian red blood cells. Scarfi et al. (1997) showed enhanced micronuclei formation in lymphocytes of patients with Turner’s syndrome (only one X chromosome) but no change in micronuclei formation when the lymphocytes were exposed to sine waves (Scarfi et al., 1996). Takahashi et al. (1986) monitored thymidine incorporation in Chinese hamster cells and explored the influence of pulse frequency (two windows of enhancement seen), pulse width (one window of enhancement seen) and intensity (two windows of enhancement seen followed by a reduction in incorporation). Ubeda et al. (1983) showed the influence of difference rise and fall times of pulsed waves on chick embryo development.

It is important to note that the frequency spectrum of pulsed waves can be represented by a sum of sine waves which, to borrow a chemical analogy, would represent a mixture or a soup of chemicals, anyone of which could be biologically active. Risk assessment and exposure limits have been established for specific chemicals or chemical classes of compounds that have been shown to cause undesirable biological effects. Risk assessors and the general public are sophisticated enough to recognize that it is impossible to declare all chemicals safe or hazardous; consider the difference between food and poisons, both of which are chemicals. A similar situation occurs for EMF; it is critical to determine which combinations of EMF conditions have the potential to cause biological harm and which do not.

Obviously, pulse wave exposures represent an entire genre of exposure conditions, with additional difficulty for exact independent replication of exposures, and thus of results, but with increased opportunities for the production of biological effects. Current standards were not developed with explicit knowledge of these additional consequences for biological responses.

F. Mechanisms

Two recent papers have the possibility of advancing understanding in this research area. Chiabrera et al. (2000) created a theoretical model for EMF effects on an ion's interaction with protein that includes the influence of thermal energy and of metabolism. Before this publication, theoreticians assumed that biological effects in living systems could not occur if the electric signal is below the signal caused by thermal noise, in spite of experimental evidence to the contrary. In this paper, the authors show that this limitation is not absolute, and that different amounts of metabolic energy can influence the amount and parametric response of biological systems to EMF. The second paper, by Marino et al. (2000), presents a new analytical approach to examine endpoints in systems exposed to EMF. The authors, focusing on exposure-induced lyphoid phenotypes, report that EMF may not cause changes in mean values of endpoints, but rather in variances in those same endpoints. They provide further evidence using immunological endpoints from exposed and sham treated mice (Marino et al., 2001a, 2001b, 2001c). Additional research has emerged from this laboratory on EMF-induced animal and human brain activity changes that provides more evidence for the value of their research approach (Marino et al., 2002, 2003, 2004; Carrubba et al., 2006, 2007a, 2007b). *It is apparent that much remains to be examined and explained in EMF biological effects research through more creative methods of analysis than have been used before. The models described above need to be incorporated into risk assessment determinations.*

IV. Problems with Segregation of Effects by Artificial Frequency Bands that Ignore Modulation

One fundamental limitation of most reviews of EMF biological effects is that exposures are segregated by the physical (engineering/technical) concept of frequency bands favored by the engineering community. This is a default approach that follows the historical context established in the past by the incremental addition of newer technologies that generate increasingly higher frequencies. However, this approach fails to consider unique responses from biological systems that are widely reported at various combinations of frequencies, modulations and intensities.

When common biological responses are observed without regard for the particular, engineering-defined EMF frequency band in which the effects occur, this reorganization of the results can highlight the commonalities in biological responses caused by exposures to EMF across the different frequently bands. An attempt to introduce this concept to escape the limitations of the engineering-defined structure occurred with the development of the 1986 NCRP radiofrequency exposure guidelines because published papers from the early 1970s to the mid 1980s (to be discussed below) demonstrated the need to include amplitude modulation as a factor in setting of maximum exposure limits. The 1986 NCRP guideline was the one and only risk evaluation that included an exception for modulated fields.

The current situation argues strongly for a change in the way risk assessment is conducted, especially for the last 15 to 20 years. Unfortunately, subsequent risk evaluations did not follow the NCRP example, but returned to the former engineering-defined analysis conditions, in part because scientists who reported non-thermal effects were not placed on the review committees, and in the terms of Slovic (1999) "Risk assessment is inherently subjective and represent a blend of science and judgment with important psychological, social, cultural, and political factors. … Whoever controls the definition of risk controls the rational solution to the problem at hand. … Defining risk is thus an exercise in power." It appears that by excluding scientists experienced with producing non-thermal biological effects, the usually sound judgment by the selected committees was severely limited in its breadth-of-experience, thereby causing the members to retreat to their own limited areas of expertise when forced to make judgments, as described by Slovic (1999), "Public views are also influenced by worldviews, ideologies, and values; so are scientists' views, particularly when they are working at limits of their expertise." The current practice of segregating scientific investigations (and resulting public health limits) by artificial divisions of frequency dramatically dilutes the impact of the basic science results, thereby reducing and distorting the weight of evidence in any evaluation process (see evaluations of bias by Havas 2000, referring to NRC 1997 compared to NIEHS 1998 and NIEHS 1999).

A. Suggested Research

Are there substitute approaches that would improve on the health-effects evaluation situation? As mentioned above, it may be useful in certain cases to develop a biologically based clustering of the data to focus on and enrich understanding of certain aspects of biological responses. Some examples to consider for biological clustering include: 1) EMF features, such as frequency and intensity inter-dependencies, 2) common cofactors, such as the earth's magnetic field or co-incident application of chemical agents to perturb and perhaps sensitize the biological system to EMF, or 3) physiological state of the biological specimen, such as age or, sensitive sub-populations, including genetic predisposition (Fedrowitz et al., 2004, 2005).

To determine if this approach has merit, one could combine reports of biological effects found in the ELF (including sub-ELF) band with effects found in the RF band when the RF exposures are amplitude modulated (AM) using frequencies in the ELF band. The following data should be used: 1) human response time changes under ELF exposure (Hamer, 1968), 2) monkey response time and EEG changes under ELF exposure (Gavalas et al., 1970; Gavales-Medici & Day-Magdaleno, 1976), 3) cat brain EEG, GABA and calcium ion changes induced by ELF and AM-RF (Kaczmarek and Adey, 1973, 1974; Bawin et al. 1973), 4) calcium ion changes in chick brain tissue under ELF and AM-RF (Bawin et al., 1975, 1976, 1978a, 1978b; Sheppard et al., 1979; Joines and Blackman et al., , 1981a, 1981b, 1986; Blackman et al., 1979, 1980a, 1980b, 1981, 1982, 1985, 1988a, 1988b, 1989, 1990), and 5) calcium changes under AM-RF in brain cells in culture (Dutta et al., 1984, 1989, 1992) and in frog heart under AM-RF (Schwartz et al., 1990). The potential usefulness of applying biological clustering in the example given above even though AM is used, is that the results may have relevance to assist in the examination of some of the effects reportedly caused by cellular phone exposures which include more complex types of modulation of RF. This suggestion is reasonable because three groups have recently reported human responses to cell phone emissions that include changes in reaction times (Preece et al., 1998, 1999; Koivisto et al. 2000a, 2000b; Krause et al., 2000a, 2000b) or to brain wave potentials that may be associated with reaction time changes (Freude et al., 1998, 2000).

The papers described above, published in the 1960s through 1991, foreshadowed the more recent publications in 1999 and 2000 showing response time changes, or associated measures, in human subjects during exposure to cell phone-generated radiation (although none of the earlier studies was acknowledged in these recent reports on cognition and reaction time). Without guidance from this extensive earlier work, the development of the mechanistic bases for non-thermal effects from EMF exposures will be substantially delayed.

V. Conclusions

- There is substantial scientific evidence that some modulated fields (pulsed or repeated signals) are bioactive, which increases the likelihood that they could have health impacts with chronic exposure even at very low exposure levels. Modulation signals may interfere with normal, non-linear biological processes.

- Modulation is a fundamental factor that should be taken into account in new public safety standards; at present it is not even a contributing factor.

- To properly evaluate the biological and health impacts of exposure to modulated RFR (carrier waves), it is also essential to study the impact of the modulating signal (lower frequency fields or ELF-modulated RF).

- Current standards have ignored modulation as a factor in human health impacts, and thus are inadequate in the protection of the public in terms of chronic exposure to some forms of ELF-modulated RF signals.

- The current IEEE and ICNIRP standards are not sufficiently protective of public health with respect to chronic exposure to modulated fields (particularly new technologies that are pulse-modulated and heavily used in cellular telephony).

- The collective papers on modulation appear to be omitted from consideration in the recent WHO and IEEE science reviews. This body of research has been ignored by current standard setting bodies that rely only on traditional energy-based (thermal) concepts.

- More research is needed to determine which modulation factors, and combinations are bioactive and deleterious at low intensities, and are likely to result in disease-related processes and/or health risks; however this should not delay preventative actions supporting public health and wellness.

- If signals need to be modulated in the development of new wireless technologies, for example, it makes sense to use what existing scientific information is available to avoid the most obviously deleterious exposure parameters and select others that may be less likely to interfere with normal biological processes in life.

- The current membership on Risk Assessment committees needs to be made more inclusive, by adding scientists experienced with producing non-thermal biological effects.

- The current practice of segregating scientific investigations (and resulting public health limits) by artificial divisions of frequency needs to be changed because this approach dramatically dilutes the impact of the basic science results and eliminates consideration of modulation signals, thereby reducing and distorting the weight of evidence in any evaluation process.

Disclaimer: the opinions expressed in this text are those of its author, and are not necessarily those of his employer.

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SECTION 15

Evidence for Disruption by Modulation Role of Physical and Biological Variables in Bioeffects of Non-Thermal Microwaves for Reproducibility, Cancer Risk and Safety Standards

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ABSTRACT

Diverse biological responses to non-thermal (NT) microwaves (MW), including adverse health effects related to increased cancer risk, have been studied by multiple research groups all over the world. In approximately half of these studies, no any effects were found (negative studies), while the other half reported the NT MW effects (positive studies). This fact is often referred to as non-reproducibility of the NT MW effects. In most cases, such a conclusion is based on comparing studies, which significantly differ in important biological and physical variables/parameters. The aim of this chapter is to provide an overview of the complex dependence of the NT MW effects on various physical and biological parameters, which must be controlled in replication studies. To the aim of this paper, all studies available to the author, which included analysis of different variables/parameters and reported some positive NT MW response to be a reference for analyzing its dependence on physical and biological parameters, were included. Selection criteria included relevant experimental design, methodological quality and statistical analysis. Besides dependencies on carrier frequency, modulation, genotype, physiological traits, presence of radical scavengers and antioxidants, reported by many research groups, the emerging data suggest dependencies of the NT MW effects on polarization, intermittence and coherence time of exposure, static magnetic field, electromagnetic stray fields, sex, age, individual traits, cell density during exposure. This overview provides clear evidence that in most cases, the references to non-reproducibility of the NT MW effects are not correct. Unfortunately, most reviews and panels in the field do not include analysis of various biological variables and physical parameters when comparing the data on the NT MW effects from different studies. As result, misleading conclusion is often made that MW at NT levels produce no “reproducible” effects. Our analysis suggests that different (bandwidth, frequency, modulation, polarization) NT MW signals should be considered as separate agents in setting the safety standards. The data also indicate that duration of exposure may be as important as power density (PD) and specific absorption rate (SAR), and, therefore, the "dose" and duration of exposure should also be considered in safety standards along with PD/SAR. Further evaluation of the dependencies of NT MW effects on biological and physical variables/parameters are needed for understanding the mechanisms by which NT MW affect biological systems, planning *in vivo* and epidemiological studies, setting the safety standards, and minimizing the adverse effects of MW from mobile communication.

Keywords: non-thermal effects of microwaves, mobile (cellular) phones, safety standards.

List of Abbreviations:

Anomalous viscosity time dependence (AVTD); blood-brain barrier (BBB); catalase (CAT); Digital Enhanced (former European) Cordless Telecommunications (DECT); circularly polarized (CP); continuous wave (CW); Digital Advanced Mobile Phone System (DAMPS); discontinuous transmission (DTX); electroencephalographic (EEG); electromagnetic field (EMF); embryonic stem (ES) cells; ethidium bromide (EtBr); extremely low frequency (ELF); Gaussian Minimum Shift Keying (GMSK); Ginkgo biloba (Gb); Global System for Mobile Communication (GSM); glutathione peroxidase (GSH-Px); International Commission for Non-Ionizing Radiation Protection (ICNIRP); linearly polarized (LP); malondialdehyde (MDA); micronucleus (MN) assay; microwaves (MWs); N-acetyl-beta-d-glucosaminidase (NAG); nitric oxide (NO); non-thermal (NT); ornithine decarboxylase (ODC); phorbol ester 12-myristate 13-acetate (PMA); phosphorylated H2AX histone (γ-H2AX); power density (PD); regional cerebral blood flow (rCBF); Russian National Committee on Non-Ionizing Radiation Protection (RNCNIRP); specific absorption rate (SAR); static magnetic field (SMF); superoxide dismutase (SOD); Time Division Multiple Access (TDMA); tumor suppressor p53 binding protein 1 (53BP1); ultraviolet (UV); Universal Mobile Telecommunications System (UMTS).

I. THERMAL VERSUS NON-THERMAL EFFECTS

Exposures to electromagnetic fields vary in many parameters: power (specific absorption rate, incident power density), wavelength/frequency, near field/far field, polarization (linear, circular), continues wave (CW) and pulsed fields (that include variables such as pulse repetition rate, pulse width or duty cycle, pulse shape, pulse to average power, etc.), modulation (amplitude, frequency, phase, complex), static magnetic field (SMF) and electromagnetic stray fields at the place of exposure, overall duration and intermittence of exposure (continuous, interrupted), acute and chronic exposures. With increased absorption of energy, so-called thermal effects of microwaves (MW) are usually observed that deal with MW-induced heating. Specific absorption rate (SAR) or power density (PD) is a main determinate for thermal MW effects. Several other physical parameters of exposure have been reported to be of importance for so-called non-thermal (NT) biological effects, which are induced by MW at intensities well below any measurable heating (Grundler, Jentzsch et al. 1988; Iskin 1990; Devyatkov, Golant et al. 1994; Pakhomov, Akyel et al. 1998; Adey 1999; Belyaev, Shcheglov et al. 2000; Betskii, Devyatkov et al. 2000; Banik, Bandyopadhyay et al. 2003; Grigoriev, Stepanov et al. 2003; Grigoriev 2004; Lai 2005; Belyaev 2010; Cifra, Fields et al. 2011) (Pakhomov and Murphy 2000).

Most often, current safety standards are based on thermal MW effects observed in short-term (acute) exposures. On the other hand, NT MW effects, especially those induced during prolonged (chronic) exposures, are accepted and taken into account for setting the national safety standards in some countries such as Russia (Grigoriev, Stepanov et al. 2003; Grigoriev 2004; Grigoriev, Nikitina et al. 2005). It should be noted that, in contrast to the ICNIRP (International Commission for Non-Ionizing Radiation Protection) safety standards (ICNIRP 1998) which are based on the acute thermal effects of MW, the standards adopted by the Russian National Committee on Non-Ionizing Radiation Protection (RNCNIRP) are based on experimental data from chronic (up to 4 month) exposures of animals to MW at various physical parameters including intensity, frequency and modulation, obtained from research performed in the former Soviet Union (Grigoriev, Stepanov et al. 2003; Grigoriev 2004; Grigoriev, Nikitina et al. 2005).

Since setting the current safety standards, the situation with exposure of the general population to MW has changed significantly. Nowadays, most of the human population is chronically exposed to MW signals from various sources including mobile phones and base stations. These exposures are characterized by low intensities, varieties and complexities of signals, and long-term durations of exposure that are comparable with a lifespan. So far, the "dose" (accumulated absorbed energy that is measured in radiobiology as the dose rate multiplied by exposure time) is not adopted for the MW exposures and SAR or PD is usually used for guidelines. To what degree SAR/PD can be applied to the nowadays NT MW chronic exposures is not known and the current state of research demands reevaluation of the safety standards (Grigoriev, Nikitina et al. 2005).

The literature on the NT MW effects is very broad. About half of available experimental studies report non-thermal biological effects of microwaves (Huss, Egger et al. 2007). There are four lines of evidence for the NT MW effects: (1) altered cellular responses in laboratory *in vitro* studies and results of chronic exposures *in vivo* studies (Grigoriev, Stepanov et al. 2003; Lai 2005; Cook, Saucier et al. 2006); (2) results of medical application of NT MW in the former Soviet Union countries (Sit'ko 1989; Devyatkov, Golant et al. 1994; Betskii, Devyatkov et al. 2000; Pakhomov and Murphy 2000; Pakhomov and Murphy 2000); (3) hypersensitivity to electromagnetic fields (EMF) ; (4) epidemiological studies suggesting increased cancer risks from using mobile phones longer than 10 years (Kundi, Mild et al. 2004; Lonn, Ahlbom et al. 2004; Hardell, Eriksson et al. 2005).

The first data on the NT effects of MW in so-called millimeter range (wavelength 1-10 mm in vacuum) was obtained by Vilenskaya and co-authors (Vilenskaya, Smolyanskaya et al. 1972) and Devyatkov (Devyatkov 1973). Highly resonant effects of ultra-weak MW (near 70 GHz) on the induction of λ-phage were first established by Webb (Webb 1979), and subsequently corroborated (Lukashevsky and Belyaev 1990). In these and subsequent studies the observed spectra of MW action were found to have the following common properties: (*1*) the MW effects were strongly dependent on the frequency (frequency windows), (*2*) there was an associated power (intensity) threshold below which no effect was observed, and above which the effects of exposure depended only weakly on power over several orders of magnitude (so-called S-shaped or sigmoid dependence), (*3*) the occurrence of MW effects depended on the duration of exposure, a certain minimum duration of exposure was necessary for an effect to manifest itself. These important regularities of the NT MW effects have previously been reviewed (Postow and Swicord 1986; Grundler, Jentzsch et al. 1988; Golant 1989; Iskin 1990; Belyaev 1992; Devyatkov, Golant et al. 1994; Pakhomov, Akyel et al. 1998; Hyland 2000; Pakhomov and Murphy 2000).

The first investigations of the NT MW effects at lower frequency ranges were performed by several research groups in USSR (Presman, IuI et al. 1961; Presman 1963) and in USA by Frey (Frey 1967; Frey 1974), Blackman and colleagues (Blackman, Benane et al. 1980; Blackman, Benane et al. 1980; Joines and Blackman 1980) and Adey and colleagues (Adey, Bawin et al. 1982; Lin-Liu and Adey 1982). These groups found dependence of the NT MW effects on modulation. The effect of pulse-modulated MW was related to peak power, whereas average power was found to be relatively unimportant (Frey 1974). Frequency dependence of the MW effects have been reported (Frey 1974).

Since that time, other groups have confirmed and extended the main findings of these pioneering studies. Below, survey of recent studies, which evaluate dependence of the NT MW effects on physical parameters and biological variables, is provided.

II. FREQUENCY DEPENDENCE AND FREQUENCY WINDOWS

The effects of NT MW on DNA repair in *E. coli* K12 AB1157 were studied by the method of anomalous viscosity time dependence (AVTD) (Belyaev, Alipov et al. 1992; Belyaev, Alipov et al. 1992). The AVTD method is a sensitive technique to detect changes in conformation of nucleoids/chromatin induced by either genotoxic or stress factors (Belyaev and Harms-Ringdahl 1996; Belyaev, Shcheglov et al. 1996; Belyaev, Alipov et al. 1997; Sarimov, Malmgren et al. 2004; Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005). Significant inhibition of DNA repair was found when X-ray-irradiated cells were exposed to MW within the frequency ranges of 51.62-51.84 GHz and 41.25-41.50 GHz. The effects were observed within two “frequency windows”, both displaying a pronounced resonance character with the resonance frequencies of 51.755 GHz and 41.32 GHz, respectively (Belyaev, Alipov et al. 1992; Belyaev, Alipov et al. 1992). Of note, these MW effects were observed at PD well below any thermal effects and could not be accounted for by heating. The frequency windows of resonance type have often been termed “resonances” as also will be used below.

The resonance frequency of 51.755 GHz was stable within the error of measurements, ±1 MHz with decreasing the PD from $3\cdot10^{-3}$ to 10^{-19} W/cm^2 (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1996). At the same time, the half-width of the resonance decreased from 100 MHz to 3 MHz revealing an extremely sharp dependence on frequency ($Q \sim 10^4$). This sharp narrowing of the 51.755 GHz resonance with decreasing the PD from $3\cdot10^{-3}$ to 10^{-7} W/cm^2 followed by an emergence of new resonances, 51.675±0.001, 51.805±0.002, and 51.835±0.005 GHz (Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997). The half-widths of all these resonances including the main one, 51.755±0.001 GHz, were about 10 MHz at the PD of 10^{-10} W/cm^2. These data were interpreted in the framework of the model of electron-conformational interactions as a splitting of the main resonance 51.755 GHz by the MW field (Belyaev, Shcheglov et al. 1996).

The MW effects were studied at different PD and several frequencies around the resonance frequency of 51.675 GHz (Shcheglov, Belyaev et al. 1997). This resonance frequency was found to be stable, ±1 MHz, within the PD range of 10^{-18} - 10^{-8} W/cm^2. Along with disappearance of the 51.675 GHz resonance response at the sub-thermal PD of 10^{-6} - 10^{-3} W/cm^2, a new resonance effect arose at 51.688±0.002 GHz (Shcheglov, Belyaev et al. 1997). This resonance frequency was also stable within the PD range studied.

Taken together, the data on NT MW effects on chromatin (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997) suggested a sharp rearrangement of the frequency spectra of MW action, which was induced by the sub-thermal MW (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997). The half-widths of all three resonances depended on PD, changing either from 2-3 MHz to 16-17 MHz (51.675 GHz and 51.668 GHz resonances) or from 2-3 MHz to 100 MHz (51.755 GHz resonance) (Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997). The data indicated also that dependencies of half-width on PD might vary for different resonance frequencies.

Significant narrowing in resonance response with decreasing PD has been found when studying the growth rate in yeast cells (Grundler 1992) and chromatin conformation in thymocytes of rats (Belyaev and Kravchenko 1994). In the Gründler’s study, the half-width of the resonance (near 41 GHz) decreased from 16 MHz to 4 MHz as PD decreased from 10^{-2} W/cm^2 to 5 pW/cm^2 (Grundler 1992).

Thus, the results of studies with different cell types indicate that narrowing of the resonance window upon decrease in PD is one of the general regularities in cell response to NT MW. This regularity suggests that many coupled oscillators are involved non-linearly in the response of living cells to NT MW as has previously been predicted by Fröhlich (Frohlich 1968).

Gapeev et al. studied effects of MW exposure (frequency range 41.75-42.1 GHz, frequency increment 50 MHz, PD 240 $\mu W/cm^2$) on the respiratory burst induced by calcium ionophore A23187 and phorbol ester 12-myristate 13-acetate (PMA) in the peritoneal neutrophils of mice (Gapeev, Safronova et al. 1996; Gapeyev, Safronova et al. 1997). MW inhibited the respiratory burst. MW effect displayed resonance-like dependence on frequency, the resonance frequency and half-width of the resonance being 41.95 GHz and 160 MHz, respectively (Q= 260) (Gapeev, Safronova et al. 1996; Gapeyev, Safronova et al. 1997). In other studies, Gapeev et al. analyzed acute zymosan-induced paw edema in mice (Gapeyev, Mikhailik et al. 2008; Gapeyev, Mikhailik et al. 2009). MW exposure of animals at the PD of 0.1 mW/cm^2 resulted in decrease of the paw edema that was frequency-dependent in the range of 42-43 GHz.

Based on the extrapolation from the data obtained in the extremely high frequency range (30-300 GHz), the values for half-width of resonances at the frequency range of mobile phones (0.9–2 GHz) were estimated to be 1-10 MHz (Sarimov, Malmgren et al. 2004). Effects of GSM (Global System for Mobile Communication) MW on chromatin conformation and 53BP1 (tumor suppressor p53 binding protein 1)/γ-H2AX (phosphorylated H2AX histone) DNA repair foci in human lymphocytes were studied in this frequency range (Sarimov, Malmgren et al. 2004; Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005; Belyaev, Markova et al. 2009). These MW effects depended on carrier frequency (Sarimov, Malmgren et al. 2004; Markova, Hillert et al. 2005; Belyaev, Markova et al. 2009). This dependence was replicated in independent experiments with lymphocytes from twenty six healthy and hypersensitive persons (Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005; Belyaev, Markova et al. 2009).

Tkalec and colleagues exposed duckweed (*Lemna minor L.*) to MW at the frequencies of 400, 900, and 1900 MHz (Tkalec, Malaric et al. 2005). The growth of plants exposed for 2 h to a 23 V/m electric field of 900 MHz significantly decreased in comparison with the control, while an electric field of the same strength but at 400 MHz did not have such effect. A modulated field at 900 MHz strongly inhibited the growth, while at 400 MHz modulation did not influence the growth significantly. At both frequencies, a longer exposure mostly decreased the growth and the highest electric field (390 V/m) strongly inhibited the growth. Exposure of plants to lower field strength (10 V/m) for 14 h caused a significant decrease at 400 and 1900 MHz while 900 MHz did not influence the growth. Peroxidase activity in exposed plants varied, depending on the exposure characteristics.

Observed changes were mostly small, except in plants exposed for 2 h to 41 V/m at 900 MHz where a significant increase (41%) was found. The authors concluded that MW might influence plant growth and, to some extent, peroxidase activity. However, the effects of MW strongly depended on the characteristics of the field exposure such as frequency and modulation. These dependences were replicated in further studies (Tkalec, Malaric et al. 2007; Tkalec, Malaric et al. 2009).

Remondini et al. analyzed changes in gene expression in human EA.hy926 endothelial cells using gene microarrays (Remondini, Nylund et al. 2006). Cells were exposed to MW (SAR 1.8-2.5 W/kg, 1 h exposure) either at 900-MHz GSM Basic mode or 1800-MHz GSM Basic mode. Exposure to 900 MHz resulted in up-regulation in 22 genes and down-regulation in 10 genes. No significant change in gene expression was observed after exposure to 1800 MHz.

III. NON-LINEARITY: SIGMOID INTENSITY DEPENDENCES AND POWER WINDOWS

Devyatkov with colleagues have found and published in Russian that wide variety of NT MW effects *in vitro* and *in vivo* display sigmoid dependence on intensity above certain intensity thresholds (Devyatkov 1973).

In English literature, one of the earliest observation of threshold in response to NT MW was published by Frey (Frey 1967). In this study, the threshold of 30 µW/cm2 was found in the study by Frey on Brain stem evoked responses to RF in cats (Frey 1967). This value was 4 orders of magnitude lower then intensities needed to cause internal body temperature increase.

In their pioneering study on blood-brain barrier (BBB) permeability, Oscar and Hawkins exposed rats to MW at 1.3 GHz and analyzed BBB permeability by measuring uptake of several neutral polar substances in certain areas of the brain (Oscar and Hawkins 1977). A single, 20 min exposure, to continuous wave (CW) MW increased the uptake of D-mannitol at average power densities of less than 3 mW/ cm^2. Increased permeability was observed both immediately and 4 h after exposure, but not 24 h after exposure. After an initial rise at 0.01 mW/ cm^2, the permeability of cerebral vessels to saccharides decreased with increasing microwave power at 1 mW/cm^2. Thus, the effects of MW were observed within the power window of 0.01- 0.4 mW/cm^2. The findings on "power windows" for BBB permeability have been subsequently corroborated by the group of Persson and Salford (Salford, Brun et al. 1994; Persson, Salford et al. 1997). In their recent study, the effects of GSM MW on the permeability of the BBB and signs of neuronal damage in rats were investigated using a real GSM programmable mobile phone in the 900 MHz band (Eberhardt, Persson et al. 2008). The rats were exposed for 2 h at an SAR of 0.12, 1.2, 12, or 120 mW/kg.

Albumin extravazation and also its uptake into neurons increased after 14 d. The occurrence of dark neurons in the rat brains increased later, after 28 d. Both effects were seen already at 0.12 mW/kg with only slight increase, if any, at higher SAR values.

Sigmoid intensity dependences and power windows for the NT MW effects were observed in many other studies as previously reviewed (Postow and Swicord 1986; Grundler, Jentzsch et al. 1988; Golant 1989; Iskin 1990; Devyatkov, Golant et al. 1994; Blackman 2009).

Since 1980, there have been numerous reports of biological effects that show intensity "windows", that is, regions of intensity that cause changes surrounded by higher and lower intensities that show no effects from exposure, see for review (Blackman 2009). These results mean that lower intensity is not necessarily less bioactive, or less harmful.

Olcerst at al have reported that MW-induced increase in rubidium passive efflux did not increase monotonically with absorbed power (Olcerst, Belman et al. 1980). In fact, the highest exposure (SAR 390 mW/g) resulted in an increase, not statistically different from the lowest exposure level (SAR 100 mW/g) For sodium ions, at the greatest SAR of 390 mW/g, the effect was the smallest (Olcerst, Belman et al. 1980).

The data obtained in experiments with *E coli* cells and rat thymocytes provided new evidence for sigmoid type of PD dependence and suggested that, similar to ELF effects, MW effects may be observed within specific "intensity windows" (Belyaev, Shcheglov et al. 1992; Belyaev and Kravchenko 1994; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997). The most striking example of the sigmoid PD dependence was found at the resonance frequency of 51.755 GHz (Belyaev, Shcheglov et al. 1996). When exposing *E. coli* cells at the cell density of $4 \cdot 10^8$ cell/ml, the effect reached saturation at the PD of 10^{-18}-10^{-17} W/cm^2 and did not change up to PD of 10^{-3} W/cm^2. In these experiments, the direct measurements of PD below 10^{-7} W/cm^2 were not available and lower PD was obtained using calibrated attenuators. Therefore, some uncertainty in the evaluation of the lowest PD was possible. The background MW radiation in this frequency range has been estimated to be 10^{-21}-10^{-19} W/m^2/Hz (Kolbun and Lobarev 1988). Based on the experimentally determined half-width of the 51.755 GHz resonance, 1 MHz (Belyaev, Shcheglov et al. 1996), the background PD was estimated as 10^{-19}-10^{-17} W/cm^2 within the 51.755 GHz resonance. The resonance MW effects on *E. coli* cells were observed at the PD very close to the estimated background value (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997; Shcheglov, Alipov et al. 2002). These data suggested that the PD dependence of MW effect at the specific resonance frequencies might have intensity threshold just slightly above the background level. Dependence of the MW effect on PD at one of the resonance frequencies, 51.675 GHz, had the shape of "intensity window" in the PD range from 10^{-18} to 10^{-8} W/cm^2 (Shcheglov, Belyaev et al. 1997). It is interesting, that no MW effect at this resonance frequency was observed at sub-thermal and thermal PD. This type of PD dependence has supported hypothesis about possible rearrangement of the frequency MW spectra action by the MW field (Belyaev, Shcheglov et al. 1996). The position of the PD window varied between different resonance frequencies and depended on cell density during exposure of cells (Shcheglov, Belyaev et al. 1997). Despite some uncertainty in the evaluation of PD at the levels below 10^{-7} W/cm^2 in the referred studies the data indicated that NT MW at the resonance frequencies may result in biological effects at very low intensities comparable with intensities from base stations and other MW sources used in mobile communication.

Gapeev et al. have studied dependence of the MW effects at the resonance frequency of 41.95 GHz on the respiratory burst induced by calcium ionophore A23187 and PMA in the peritoneal neutrophils of mice (Gapeev, Safronova et al. 1996; Gapeyev, Safronova et al. 1997). Inhibitory effects of MW exposure has been observed at the PD of 0.001 mW/cm^2 and displayed sigmoid dependence on PD at higher power densities (Gapeev, Safronova et al. 1996; Gapeyev, Safronova et al. 1997). In other study, Gapeev et al. analyzed acute zymosan-induced paw edema in mice (Gapeyev, Mikhailik et al. 2009). MW exposure of animals at the frequency of 42.2GHz and exposure duration of 20 min decreased the paw edema. Sigmoid dependence of this effect on PD has been obtained with a maximum at the PD of 0.1 mW/cm^2.

French et al. exposed human astrocytoma cells to EMR at 835 MHz at a power density of either 40 mWcm^2 or 8.1 mWcm^2 (French, Donnellan et al. 1997). Lower power signal was more potent than high power signal. At the lower power density, it was observed that the rate of DNA synthesis decreased, and that the cells flattened and spread out in comparison to unexposed cultures. At higher power density there were no effects seen on cell proliferation, but alteration in cell morphology included increased cell spreading and also the appearance of actin-containing blebs at localized sites on the membrane. It was hypothesized that 835 MHz radiation at low power density may be affecting a signal transduction pathway involved in cell proliferation.

Sigmoid dependence of the negative impact of mobile phone usage on semen quality in human males was found in recent study analyzing motility, vitality, ROS generation by the whole cell, ROS generation by the mitochondria, oxidative DNA damage and DNA fragmentation (De Iuliis, Newey et al. 2009). Specifically, all of the responses examined showed an extremely rapid change at low SAR exposures that then reached a plateau at a point where around 30% of the sperm population was affected.

Hintzsche et al. have recently reported sigmoid dependence on PD in the range up to 4.3 mW/cm^2 for non-thermal effects of MW on mitotic spindle in human-hamster hybrid cells (Hintzsche, Jastrow et al. 2011).

Sun et al. have investigated the effects of exposure to a 1.8-GHz radiofrequency radiation (RFR) at different intensities on epidermal growth factor (EGF) receptor clustering and phosphorylation in human amniotic (FL) cells (Sun, Shen et al. 2012). The results showed that exposure to RFR at specific absorption rate (SAR) of 0.5, 1.0, 2.0, or 4.0 W/kg for 15 min significantly induced EGF receptor clustering and enhanced phosphorylation of the tyrosine-1173 residue in FL cells. The RFR effect displayed a sigmoid-dependence on SAR with a prominent plateau in the range of 0.5-4 W/kg and a threshold below 0.5 W/kg.

It should be mentioned that almost all biophysical mechanisms, which have previously been proposed to account for NT MW effects, predict thresholds in dependence of these effects in intensity (Grundler, Jentzsch et al. 1988; Golant 1989; Iskin 1990; Devyatkov, Golant et al. 1994; Golo 2005; Matronchik and Belyaev 2008).

To conclude, since 1970, there have been numerous reports of biological effects that show thresholds, sigmoid dependence of the NT MW effects on intensity and also "power windows", that is, regions of intensity that cause changes surrounded by higher and lower intensities that show no effects from exposure. These results mean that: (i) lower intensity is not necessarily less bioactive, or less harmful;(ii)the NT effects may be observed at intensities above thresholds which are very close to background levels and similar to intensities from base stations.

IV. DOSE AND DURATION OF EXPOSURE

So far, the "dose" (accumulated absorbed energy that is measured in radiobiology as the dose rate multiplied by exposure time) is not adopted for the MW exposures and PD or SAR (dose rate analog in radiobiology) is usually used for guidelines. To what degree SAR/PD can be applied to the nowadays NT MW chronic exposures is not exactly known and the current state of research demands reevaluation of the safety standards (Grigoriev, Nikitina et al. 2005).

Based on mechanistic consideration of the NT MW effects, Frey has suggested that the toxicology model used by investigators was not the appropriate model on which to design MW experiments (Frey 1993). With chemical substance in a toxicology model, a dose-response relationship is usually observed: the greater the dose, the greater the effect. In analogy with toxicology, MW experiments tended to be designed with high doses and with little regard for other parameters such as modulation and frequency. This might be one reason why many MW studies yielded so little useful information (Frey 1993).

The role of exposure duration in combination with dose rate/SAR for appearance and persistence of the NT MW effects have been analyzed by many research groups using various end-points.

Koveshnikova et al. exposed rats to pulsed MW (carrier frequency 3 GHz, pulse repetition 400 Hz, rectangular pulses of 2 μs, power flux density , PD, of 100, 500 and 2500 μW/cm2), during 60 days, 12 h/daily (Koveshnikova and Antipenko 1991) (is a determining factor 1991b). Chromosomal abreactions (CA) were analyzed in hepatocytes. Exposure was performed at three arrays of pulses so that 16, 29 or 48 arrays of pulses per 1 min were generated. The ratio of the obtained doses per animal was 1 : 1.8 : 3, correspondingly. Increased level of CA was generally observed at PD > 100 μW/cm^2. Importantly, the differences between PD disappeared when the dose per animal increased. In particular, even the PD of 100 μW/cm2 induced CA at higher absorbed doses. These data support the notion that the absorbed dose may be an important parameter for estimation of risks.

Bozhanova with co-authors reported that the effect of cellular synchronization induced by NT MW depended on duration of exposure and PD (Bozhanova, Bryukhova et al. 1987). The dependence on duration of exposure fitted to exponential function. The important observation was that in order to achieve the same synchronization of cells, the decrease in PD could be compensated by the increase in the duration of exposure.

MW exposure of *E. coli* cells and rat thymocytes at PDs of 10^{-5}-10^{-3} W/cm^2 resulted in significant changes in chromatin conformation if exposure was performed at resonance frequencies during 5-10 min (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992; Belyaev and Kravchenko 1994). Decrease in the MW effects due to lowering the PD by orders of magnitude down to 10^{-14}-10^{-17} W/cm^2 could be compensated by several-fold increase of exposure time to 20-40 min (Belyaev, Alipov et al. 1994). At the relatively longer duration of exposure, more then 1 h, and the lowest PD of 10^{-19} W/cm^2, the same effect was induced as at highest PDs and shorter durations (Belyaev, Alipov et al. 1994).

Kwee and Raskmark analyzed effects of MW at 960 MHz and various SARs, 0.021, 0.21, and 2.1 mW/kg on proliferation of human epithelial amnion cells (Kwee and Raskmark 1998). These authors found linear correlations between exposure time to MW at 0.021 and 2.1 mW/kg and the MW-induced changes in cell proliferation albeit no such clear correlation was seen at 0.21 mW/kg.

Peinnequin et al. have studied effects of 24 or 48 h MW 2.45 GHz exposure at non-thermal level, 5 mW/cm^2, on apoptosis in human T-cell line Jurkat clone E6-1 (Peinnequin, Piriou et al. 2000). MW affected Fas -, but neither butyrate- nor ceramide - induced apoptosis. This effect depended on exposure time and was observed only upon 48 h exposure.

Croft et al. have tested twenty-four subjects participated in a single-blind fully counterbalanced cross-over design, where both resting EEG and phase-locked neural responses to auditory stimuli were measured while a mobile phone (MP) was either operating or turned off (Croft, Chandler et al. 2002). MP exposure altered resting EEG, decreasing 1-4 Hz activity (right hemisphere sites), and increasing 8-12 Hz activity as a function of exposure duration. MP exposure also altered early phase-locked neural responses, attenuating the normal response decrement over time in the 4-8 Hz band, decreasing the response in the 1230 Hz band globally and as a function of time, and increasing midline frontal and lateral posterior responses in the 30-45 Hz band. The data have shown that active MPs affect neural function in humans and do so as a function of exposure duration.

Caraglia et al. have evaluated the in vivo effect of MW-EMF in human epidermoid cancer KB cells (Caraglia, Marra et al. 2005). It was found that MW-EMF induced time-dependent apoptosis (45% after 3 h) that was paralleled by an about 2.5-fold decrease of the expression of ras and Raf-1 and of the activity of ras and Erk-1/2.

Gapeyev et al. studied anti-inflammatory effect of low-intensity MW exposure (0.1 mW/cm^2) using the model of acute zymosan-induced footpad edema in mice (Gapeyev, Mikhailik et al. 2008). Single whole-body MW exposure of mice at the frequencies of 42.2, 51.8, and 65 GHz after zymosan injection reduced both the footpad edema and local hyperthermia. At the frequency of 42.2 GHz the effect had sigmoid dependence on exposure duration with a maximum at 20-80 min. A linear dependence on the exposure duration with significantly lower increment was observed at a 10-fold less intensity (0.01 mW/cm^2). However, this decrease in the effect was compensated by a slight increase in duration of exposure from 80 min to 120 min.

Recently, the negative impact of mobile phone usage on semen quality in human males was repeatedly found to correlate with the duration of exposure (Agarwal, Deepinder et al. 2008; Agarwal, Desai et al. 2009).

Gerner et al. exposed human fibroblats to modulated GSM 1800 MHz at 2 W/kg (Gerner, Haudek et al. 2010). While short-term exposure within 2 hours did not significantly alter the proteome, an 8-h exposure caused a significant and reproducible increase in protein synthesis. Most of the proteins found to be induced were chaperones, which are mediators of protein folding. Heat-induced proteome alterations detectable with used proteome methodology would require heating greater than 1°C. Because GSM-induced heating was less than 0.15°C, a heat-related response was excluded. These data further supported the notion that the exposure time seems to be a critical factor.

Differentiated astroglial cell cultures were exposed for 5, 10, or 20 min to either 900 MHz continuous waves or 900 MHz waves modulated in amplitude at 50 Hz (Campisi, Gulino et al. 2010). The strength of the electric field at the sample position was 10 V/m (rms). The irradiation conditions allowed the exclusion of any possible thermal effect. A significant increase in ROS levels and DNA fragmentation was found only after exposure of the astrocytes to modulated MW for 20 min. No evident effects were detected when shorter time intervals were used.

Adang et al. exposed Wistar albino rats to low-level RF during 21 months to two different microwave frequencies and exposure modes, 2 h a day, seven days a week (Adang, Remacle et al. 2009). After 14 and 18 months of exposure, the authors observed a significant increase in white blood cells and neutrophils of about 15% and 25%, respectively. Lymphocytes fell down after 18 months of exposure with about 15% compared to the sham-exposed group. No effects were observed at shorter duration of exposure. Exposure may probably have worked as a trigger and influenced the immune system, which reacted to this stressor by increasing the percentage of monocytes in the peripheral blood circulation.

Schrader et al. analysed production of spindle disturbances in FC2 cells, a human-hamster hybrid (A(L)) cell line, by MW with a field strength of 90 V/m at a frequency of 835 MHz (Schrader, Munter et al. 2008). Sigmoid dependence on time of exposure was observed with linear increase up to 30 min of exposure and saturation at longer exposures up to 2 h.

Markova et al. have found that inhibitory effect of MW on the 53BP1 foci leveled off at 1h-exposure (Markova, Malmgren et al. 2010). Human mesenchymal stem cells (MSC) and fibroblasts were expsosed to MW at GSM 915 MHz/UMTS 1947 MHz and SAR of 37/39 mW/kg. No further increase in effects was observed both in MSC and fibroblasts at prolongation of exposure to 3 h. This data are in agreement with previous results obtained in human peripheral blood lymphocytes that MW effects were the same at 1-h and 2-h exposures (Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005).

Panagopoulos and Margaritis have studied the effects of different durations of a single (continuous), daily exposure, ranging from 1 min up to 21 min, to EMF from GSM 900 MHz (Global System for Mobile telecommunications) and DCS 1800 MHz (Digital Cellular System-referred to also as GSM 1800 MHz), on the reproductive capacity of Drosophila melanogaster (Panagopoulos and Margaritis 2010). The insects were exposed to each type of radiation at intensity of about 10 μW/cm^2, corresponding to a distance of 20 or 30 cm from the antenna of a DCS 1800 or a GSM 900 mobile phone handset, respectively. The results show that the reproductive capacity decreases almost linearly with increasing exposure duration to both GSM 900 and DCS 1800 radiation, suggesting that short-term exposures to these radiations have cumulative effects. Additionally, the results show that GSM 900 MHz radiation is slightly more bioactive than DCS 1800 MHz radiation, at the same exposure durations and under equal radiation intensities.

In some studies, the prolonged MW exposures were associated with less prominent effects than shorter exposures (Nikolova, Czyz et al. 2005; Tkalec, Malaric et al. 2007; Markova, Malmgren et al. 2010). This type of dependence on exposure duration was explained by adaptation of the exposed biosystems to the MW exposure (Markova, Malmgren et al. 2010).

Esmekaya et al. exposed human peripheral blood lymphocyte to GSM modulated MW radiation at 1.8 GHz and SAR of 0.21 W/kg for 6, 8, 24 and 48 h (Esmekaya, Aytekin et al. 2011). The authors reported morphological changes in exposed lymphocytes. Longer exposure periods led to destruction of organelle and nucleus structures. Chromatin change and the loss of mitochondrial crista occurred in cells exposed to RF for 8 h and 24 h and were more pronounced in cells exposed for 48 h. RF exposure did not increase the temperature. The authors concluded that the greater damage occurred after longer periods of exposure to NT MW.

Tepe Çam and Seyhan have analyzed DNA damage in hair root cells of volunteers before and after they have used 900-MHz GSM mobile phone for 15 or 30 min. The 900-MHz GSM exposure significantly increased single-strand DNA breaks in cells of hair roots close to the position of phone at the heads of volunteers. 30 min talking by mobile phone induced more DNA damage than 15 min talking (Cam and Seyhan 2012).

Nazıroğlu et all have measured cytosolic free Ca^{2+} in human leukemia cells during 1-24 h exposure to 2.45 GHz electromagnetic radiation at the average SAR of 1.63 W/kg (Naziroglu, Cig et al. 2012). Radiation induced increase of cytosolic free Ca^{2+} concentration was time-dependent and was highest at 24-h exposure.

In some studies, prolonged MW exposures were associated with less prominent effects than shorter exposures (Nikolova, Czyz et al. 2005; Tkalec, Malaric et al. 2007; Markova, Malmgren et al. 2010). This type of dependence on exposure duration was accounted for adaptation of the exposed systems to the MW exposure. The magnitude of adaptation depends on a number of biological variables that will be considered elsewhere.

In recent German study, 24 out of 60 participants were exposed to MW from base station at a power density of < 60 μW/m², 20 participants to 60 - 100 μW/m², and 16 participants to more than 100 μW/m² (Buchner and Eger 2011). The values of the stress hormones adrenaline and noradrenaline grew significantly during the first 6 months after starting the GSM base station; the values of the precursor substance dopamine substantially decreased in this time period. The initial condition was not restored even after 1.5 years. Due to the not regulable chronic difficulties of the stress balance, the phenylethylamine levels dropped until the end of the investigation period. These effects show a dose-effect relationship.

Recently reported general indications of a dose–response relationship between chronic exposure to cellular phone MW and parotid gland malignancy indicate necessity of the dose approach at the epidemiological level (Duan, Zhang et al. 2011). For the first time in epidemiology of RF-induced tumors, Cardis et al. have used estimates of radio frequency energy deposition at the centre of tumors in the brain as a measure of MW dose (Cardis, Armstrong et al. 2011). An increased risk of glioma was seen in individuals at the highest quintile of radio frequency dose, though reduced risks were seen in the four lower quintiles. When risk was examined as a function of dose received in different time windows before diagnosis, an increasing trend was observed with increasing MW dose (for exposures 7 years or more in the past.

In conclusion, the data from different groups suggest that duration of exposure and dose may have significant role for the NT MW effects. In specially designed studies, reduction in dose rate/SAR could be compensated by prolongation of exposure time in order to achieve the same MW effect. The temporal nature of the MW effects contributes to the apparent lack of consistent results reported in the literature. Emerging epidemiology data indicate that the dose of MW exposure may correlate with the increased brain tumor risk.

V. TIME AFTER EXPOSURE

The MW effects on *E. coli* cells significantly depended on the post-exposure time (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994; Shcheglov, Alipov et al. 2002). This dependence had an initial phase of increase about 100 min post-exposure followed by a phase, which was close to a plateau, around 100 min. A trend to decrease in effect was observed at longer times up to 300 min (Belyaev, Shcheglov et al. 1993; Shcheglov, Alipov et al. 2002).

Significant MW-induced changes in chromatin conformation were observed when rat thymocytes were analyzed in-between 30-60 min after exposure to MW (Belyaev and Kravchenko 1994). This effect nearly disappeared if the cells were incubated more than 80 min between exposure and analysis.

Gapeev et al. have studied dependence of the MW effect on the function of the mouse peritoneal neutrophils in dependence on duration of exposure at the frequency of 41.95 GHz and the PD of 240 μW/cm^2 (Gapeev, Safronova et al. 1996; Gapeyev, Safronova et al. 1997). This dependence had a bell-shaped form with the maximal effects at 20 - 40 min of exposure.

In recent studies, human lymphocytes from peripheral blood of healthy and hypersensitive to EMF persons were exposed to NT MW from the GSM mobile phones (Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005). NT MW induced changes in chromatin conformation similar to those induced by heat shock, which remained up to 24 h after exposure. It was found in the same and following studies that GSM MW at the carrier frequency of 915 MHz and UMTS (Universal Mobile Telecommunications System) MW at 1947.4 MHz inhibited formation of 53BP1/γ-H2AX DNA repair foci and these adverse effects remained during 72 h after an 1-h exposure (Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005; Belyaev, Markova et al. 2009). The same group has reported that contrary to human fibroblast, which were able to adapt during chronic exposure to GSM/UMTS non-thermal MW, human stem cells did not adapt (Markova, Malmgren et al. 2010).

Jorge-Mora et al. investigated the effects of MW 2.45 GHz radiation on the paraventricular nucleus (PVN) of the hypothalamus, extracted from brains of exposed rats (Jorge-Mora, Misa-Agustino et al. 2011). Expression of c-Fos was analyzed in rats exposed once or repeatedly (ten times in 2 weeks) to MW at non-thermal SAR of 0.0776 and 0.301 W/kg. High SAR triggered an increase of the c-Fos marker 90 min or 24 h after radiation, and low SAR resulted in c-Fos counts higher than in control rats after 24 h. Repeated irradiation at 0.0776 W/kg increased cellular activation of PVN by more than 100% compared to animals subjected to acute irradiation and to repeated non-radiated repeated session control animals. The results suggest that the time of exposure to single or repeated doses of NT MW is a determining factor, though possibly not the only factor, in establishing the power levels that may produce a response.

Lu et al. have demonstrated that reactive oxygen species (ROS) plays an important role in the process of apoptosis in human peripheral blood mononuclear cell (PBMC), which is induced by the exposure to 900 MHz radiofrequency electromagnetic at the SAR of 0.4W/kg when the exposure lasts longer than two hours (Lu, Huang et al. 2012).

The data indicate that there is a time window for observation of the NT MW effects, which may be dependent on endpoint measured, cell type, duration and PD of exposure.

VI. COHERENCE TIME

MW exposure of L929 fibroblasts was performed by the group of Litovitz (Litovitz, Krause et al. 1993). MW at 915 MHz modulated at 55, 60, or 65 Hz approximately doubled ornithine decarboxylase (ODC) activity after 8 h. Switching the modulation frequency from 55 to 65 Hz at coherence times of 1.0 s or less abolished enhancement, while times of 10 s or longer provided full enhancement. These results suggested that the microwave coherence effects are remarkably similar to those observed previously with extremely low frequency (ELF) magnetic fields by the same authors.

VII. INTERMITTENCE

Diem and colleagues exposed cultured human diploid fibroblasts and cultured rat granulosa cells to intermittent and continuous MW (1800 MHz; SAR 1.2 or 2 W/kg; different modulations; during 4, 16 and 24 h; intermittent 5 min on/10 min off or continuous exposure) (Diem, Schwarz et al. 2005). Comet assay was applied to analyze DNA single- and double-strand breaks. MW-induced effects occurred after 16 h exposure in both cell types and after different mobile-phone modulations. The intermittent exposure showed a stronger effect than continuous exposure.

Remondini et al. analyzed changes in gene expression in human HL-60 leukemia cells using gene microarrays (Remondini, Nylund et al. 2006). Cells were exposed to MW (SAR 1.0-1.3 W/kg, 1800 MHz DTX mode, 24 h exposure) either continuously or intermittently, 5 min ON/5 min OFF. Gene expression was affected by intermittent exposure but not continuous exposure.

Elhag et al. investigated effect of near field EMR from GSM mobile phones on the oxidant and antioxidant status in rats (Elhag, Nabil et al. 2007). Rats were subjected to either intermittent exposure (15 min/day for four days) or acute exposure for 1 h. Significant drop in the plasma concentration of vitamin C, vitamin E, vitamin A and reduced glutathione (GSH) was observed in both exposed groups as compared to controls. EMR exposure of rats produced a significant decrease in catalase (CAT) and superoxide dismutase (SOD) activities, with the values of these activities for acute-exposure group is significantly lower than those of intermittent exposure. The authors concluded that the effects of acute exposure to mobile phones on the rat's antioxidant status is significantly higher than those of intermittent exposure of the same type of radiation.

Chavdoula et al used a 6-min daily exposure of dipteran flies, Drosophila melanogaster, to GSM-900MHz (Global System for Mobile Telecommunications) mobile phone electromagnetic radiation (EMR), to compare the effects between the continuous and four different intermittent exposures of 6 min total duration on the insect's reproductive capacity as well as on the induction of apoptosis (Chavdoula, Panagopoulos et al. 2010). It was found that intermittent exposure, similar to continuous exposure, decreases the reproductive capacity and alters the actin-cytoskeleton network of the egg chambers, another known aspect of cell death, and that this effect is due to DNA fragmentation. Intermittent exposures with 10-min intervals between exposure sessions proved to be almost equally effective as continuous exposure of the same total duration, whereas longer intervals between the exposures seemed to allow the organism the time required to recover and partly overcome the above-mentioned effects of the GSM exposure.

VIII. MODULATION

Several types of modulations used in mobile communication have previously been reviewed (Foster and Repacholi 2004; Blackman 2009; Juutilainen, Hoyto et al. 2011). In particular, the 2G signals use the Gaussian Minimum Shift Keying (GMSK) modulation, have a high coherence, extremely low frequency amplitude modulation spectra, high crest factor (pulsed signal) and a power regulation with an update in the order of seconds. In contrast, the 3G Wideband Code-Division Multiple Access (WCDMA) uses essentially Quadrature Phase Shift Keying (QPSK) modulation, has a low coherence and a broad-band extremely low frequency amplitude modulation spectrum.

While considering effect of modulation, all other parameters, which are important for appearance of biological effects induced by NT MW, should be taken into account. In particular it is useless to include in analysis the papers where no effects of NT MW were detected at all because usually these studies do not scan the parameters of exposure in wide range to enable detecting the NT MW effects. Even more importantly is to analyze separately different types of modulations because each type may result in its own specific effect. When such approach is used, clear evidence is emerging for the effects of specific modulations. For example, among three studies on cancer-relevant non-genotoxic endpoints, biological effects (apoptosis, altered cell proliferation, lipid peroxidation) were induced by GSM modulated signal but not by a CW signal (Juutilainen, Hoyto et al. 2011). All these studies involved combined exposure to RF fields and other agents, and found GSM-modulation-specific effects on apoptosis. Another example is increased power in the alpha band (8–12 Hz) of EEG, which has been consistently seen in several studies most of which have used GSM-type modulation and have found that signals with pulse modulation are more biologically active than CW fields, or that signals with higher degree of modulation (e.g., handset-like signals) are more biologically active than signals with lower degree of modulation (e.g., base station-like signals). Studies that have used only GSM-type signals have provided additional evidence for effects of modulated RF signals on human brain functions (van Rongen, Croft et al.

2009). Overall, the consistency of the positive findings indicates that there may be reproducible modulation-specific effects on the human central nervous system (Juutilainen, Hoyto et al. 2011). This result is consistent with the well-known notion that properly modulated RF may be a useful tool in experiments directed at understanding nervous system function (Frey 1967).

Using aforementioned approach, it became clear that significant body of papers where NT MW effects were observed and modulated and unmodlatlated signals were carefully compared revealed the differences. There is strong experimental evidence for the role of modulation in the diverse biological effects of NT MW both in vitro and in vivo (Lin-Liu and Adey 1982; Byus, Lundak et al. 1984; Dutta, Subramoniam et al. 1984; Byus, Kartun et al. 1988; Dutta, Ghosh et al. 1989; Veyret, Bouthet et al. 1991; Gapeev, Iakushina et al. 1997; Litovitz, Penafiel et al. 1997; Penafiel, Litovitz et al. 1997; Persson, Salford et al. 1997; d'Ambrosio, Massa et al. 2002; Huber, Treyer et al. 2002; Markkanen, Penttinen et al. 2004; Huber, Treyer et al. 2005). Examples include different types of modulation such as amplitude-, speech and phase modulations: (i) Amplitude modulation at 16 Hz, but not 60 Hz or 100 Hz, of a 450-MHz MW increased activity of ODC (Byus, Kartun et al. 1988). (ii) Speech-modulated 835-MHz MW produced no effect on ODC as compared to the typical signal from a TDMA (Time Division Multiple Access) digital cellular phone (Penafiel, Litovitz et al. 1997). (iii) Phase-modulated GSM-1800 MW (Gaussian Minimum Shift Keying, GMSK) at 1.748 GHz induced micronuclei in human lymphocytes while CW MW did not (d'Ambrosio, Massa et al. 2002).

Normal human lymphocytes were exposed for 5 days to continuous wave (CW) or pulsed wave (PW) 2450-MHz radiation at non-heating (37 degrees C) and various heating levels (temperature increases of 0.5, 1.0, 1.5, and 2 degrees C) (Czerska, Elson et al. 1992). The pulsed exposures involved 1-microsecond pulses at pulse repetition frequencies from 100 to 1,000 pulses per second at the same average SAR levels as the CW exposures. At non-heating levels, CW exposure did not affect lymphoblastoid transformation. At heating levels both conventional and CW heating enhanced transformation to the same extent and correlate with the increases in incubation temperature. PW exposure enhanced significantly transformation at non-heating levels. At heating levels PW exposure enhanced transformation to a greater extent than did conventional or CW heating. Authors concluded that PW 2450-MHz radiation acts differently on the process of lymphoblastoid transformation in vitro compared with CW 2450-MHz radiation at the same average SARs.

Bolshakov and Alexeeev used microelectrode and voltage-clamp techniques to record spontaneous electrical activity and ionic currents of Lymnea stagnalis neurons during exposure to a 900-MHz field in a waveguide-based apparatus (Bolshakov and Alekseev 1992). The field was pulse-modulated at repetition rates ranging from 0.5 to 110 pps, or it was applied as a continuous wave (CW). When subjected to pulsed waves (PW), rapid, burst-like changes in the firing rate of neurons occurred at SARs of a few W/kg. If the burst-like irregularity was present in the firing rate under control conditions, irradiation enhanced its probability of occurrence. The effect had a threshold SAR near 0.5 W/kg. CW radiation had no effect on the firing rate pattern at the same SAR. Thus, the effect was dependent on modulation. Mediator-induced, current activation of acetylcholine, dopamine, serotonin, or gamma-aminobutyric-acid receptors of the neuronal soma was not altered during CW or PW exposures and, hence, could not have been responsible for the bursting effect.

Gapeev and co-authors studied production of reactive oxygen species (ROS) in isolated peritoneal neutrophils of mice using a model of synergistic reaction of calcium ionophore A23187 and phorbol ester PMA (Gapeev, Iakushina et al. 1997; Gapeyev, Yakushina et al. 1998). MW exposure at 41.95 GHz, continuous wave mode and 50 μW/cm^2, inhibited ROS production. MW modulated with the frequency of 1 Hz resulted in stimulation of the synergistic reaction. Modulation frequencies of 0.5, 2, 4, and 8 Hz did not cause significant effects, and modulation frequencies of 0.1, 16, and 50 Hz inhibited the synergistic reaction.

In other study, Gapeev et al. analyzed acute zymosan-induced paw edema in mice (Gapeyev, Mikhailik et al. 2009). MW exposure of animals at the PD of 0.1- 0.7 mW/cm^2 and some "effective" frequencies in the range of 42-43 GHz decreased the paw edema. Application of different modulation frequencies from the range of 0.03–100 Hz to MW exposure at the effective carrier frequency of 42.2 GHz did not lead to considerable changes in the effect. In contrast, modulation of MW at the ''ineffective'' carrier frequencies of 43.0 and 61.22 GHz by frequencies from the ranges of 0.07–0.1 and 20–30 Hz resulted in a maximal anti-inflammatory effects. The results suggested a complex dependence of the anti-inflammatory action of low-intensity MW on carrier and modulation frequencies.

Capri et al. evaluated the nonthermal effects of both a 900 MHz GSM signal and a 900 MHz CW RF field at low SARs (70–76 mW/kg average) on human peripheral blood mononuclear cells (PBMCs) *in vitro* (Capri, Scarcella et al. 2004). Data obtained from cells exposed to a GSM-modulated RF field showed a slight decrease in cell proliferation when PBMCs were stimulated with the lowest mitogen concentration and a slight increase in the number of cells with altered distribution of phosphatidylserine across the membrane. Data obtained from CW-exposed cultures showed no difference with respect to sham-exposed cultures in any of the end points studied.

Huber with coauthors investigated effects of MW similar to those used in mobile communication, a "base-station-like" and a "handset-like" signal (10 g tissue-averaged spatial peak-

SAR of 1 W/kg for both conditions), on waking regional cerebral blood flow (rCBF) in 12 healthy young men (Huber, Treyer et al. 2005). The effect depended on the spectral power in the amplitude modulation of the carrier frequency such that only “handset-like” MW exposure with its stronger low-frequency components but not the “base-station-like” MW exposure affected rCBF. This finding supported previous observations of these authors (Huber, Treyer et al. 2002) that pulse modulation of MW is of importance for changes in the waking and sleep EEG, and substantiated the notion that pulse modulation is crucial for MW-induced alterations in brain physiology.

Markkanen et al. exposed cdc48-mutated *Saccharomyces cerevisiae* yeast cells to 900 or 872 MHz MW, with or without exposure to ultraviolet (UV) radiation, and analyzed apoptosis (Markkanen, Penttinen et al. 2004). Amplitude modulated (217 pulses per second) MW significantly enhanced UV induced apoptosis in cells, but no effect was observed in cells exposed to unmodulated fields at the identical time-average SAR of 0.4 W/kg that was lower than the ICNIRP safety standards.

Persson and colleagues studied effects of MW of 915 MHz as CW and pulse-modulated with different pulse power and at various time intervals on permeability of the blood-brain barrier (BBB) in Fischer 344 rats (Persson, Salford et al. 1997). Albumin and fibrinogen were demonstrated immunochemically and classified as normal versus pathological leakage. The CW-pulse power varied from 0.001 W to 10 W and the exposure time from 2 min to 960 min. The frequency of pathological rats significantly increased in all exposed rats. Grouping the exposed animals according to the level or specific absorption energy (J/kg) gave significant difference in all levels above 1.5 J/kg. The exposure was 915 MHz MW either pulse modulated at 217 Hz with 0.57 ms pulse width, at 50 Hz with 6.6 ms pulse width, or CW. The frequency of pathological rats was significantly higher in MW-exposed groups than in controls and the frequency of pathological rats after exposure to pulsed radiation was significantly less than after exposure to CW.

In a study by Lypez-Martin et al. (Lopez-Martin, Brogains et al. 2009), GSM-exposed picrotoxin-pretreated rats showed differences in clinical and EEG signs, and in c-Fos expression in the brain, in comparison to picrotoxin-treated rats exposed to an equivalent dose of unmodulated radiation. Neither MW exposure caused tissue heating, so thermal effects could be ruled out. The most marked effects of GSM MW on c-Fos expression in picrotoxin-treated rats were observed in limbic structures, olfactory cortex areas and subcortical areas, the dentate gyrus, and the central lateral nucleus of the thalamic intralaminar nucleus group. Nonpicrotoxin-treated animals exposed to unmodulated radiation showed the highest levels of neuronal c-Fos expression in cortical areas. These results suggested a specific effect of the pulse GSM modulation on brain activity of a picrotoxin-induced seizure-proneness rat model.

Luukkonen et al. investigated effects of MW at 872 MHz and relatively high SAR value (5 W/kg) on intracellular reactive oxygen species (ROS) production and DNA damage in human SH-SY5Y neuroblastoma cells. The experiments also involved combined exposure to MW and menadione, a chemical inducing intracellular ROS production and DNA damage. Both CW and a pulsed signal similar to that used in GSM mobile phones were used. Exposure to the CW radiation increased DNA breakage in comparison to the cells exposed only to menadione. Comparison of the same groups also showed that ROS level was higher in cells exposed to CW RF radiation at 30 and 60 min after the end of exposure. No effects of the GSM-like modulated signal were seen on either ROS production or DNA damage.

Hinrikus et al. (Hinrikus, Bachmann et al. 2008) evaluated the effects of MW (450 MHz) pulse-modulated at the frequencies of 7, 14 and 21 Hz on human electroencephalographic (EEG) rhythms. The field power density at the scalp was 0.16 m W/cm^2. Modulated microwaves caused an increase in the average EEG alpha (17%) and beta (7%) power but the theta rhythm remained unaffected. Increases in the EEG alpha and beta power were statistically significant during the first half-period of the exposure interval (30 s) at the modulation frequencies of 14 and 21 Hz. The authors concluded that the effect of the 450-MHz MW modulated at 7, 14 and 21 Hz varies depending on the modulation frequency.

Hoyto et al. exposed human SH-SY5Y neuroblastoma and mouse L929 fibroblast cells to MW (SAR of 5 W/kg) at 872 MHz using continuous-waves (CW) or a modulated GSM-like signal under isothermal conditions (Hoyto, Luukkonen et al. 2008). Menadione was used to induce reactive oxygen species, and tert-butylhydroperoxide (t-BOOH) was used to induce lipid peroxidation. Two statistically significant differences related to MW exposure were observed: Lipid peroxidation induced by t-BOOH was increased in SH-SY5Y (but not in L929) cells, and menadione-induced caspase 3 activity was increased in L929 (but not in SH-SY5Y) cells. Both differences were statistically significant only for the GSM-modulated signal.

Franzellitti et al. exposed human trophoblast HTR-8/SVneo cells to MW at 1.8 GHz CW and differently modulated GSM signals (GSM-217Hz, (speaking only): and GSM-Talk (34% of speaking and 66% of hearing):) during 4 - 24 h (Franzellitti, Valbonesi et al. 2008). The inducible HSP70C transcript was significantly enhanced after 24 h exposure to GSM-217 Hz signals while being reduced after 4 and 16 h exposure to GSM-Talk signal. In another study of the same group , HTR-8/SVneo cells were exposed for 4, 16 or 24 h to 1.8 GHz continuous wave (CW) and different GSM signals, namely GSM-217 Hz and GSM-Talk (intermittent exposure: 5 min field on, 10 min field off). The alkaline comet assay was used to evaluate primary DNA damages and/or strand breaks due to uncompleted repair processes in HF-EMF exposed samples. The amplitude-modulated signals GSM-217 Hz and GSM-Talk induced a significant increase in comet parameters in trophoblast cells after 16 and 24 h of exposure, while the un-modulated CW was ineffective (Franzellitti, Valbonesi et al. 2010).

Only CW RF resulted in statistically significant effect on immune system of the exposed rats (Campisi, Gulino et al. 2010). In this study, primary rat neocortical astroglial cell cultures were exposed to MW for 5, 10, or 20 min to either 900 MHz continuous waves or 900 MHz waves modulated MW in amplitude at 50 Hz using a sinusoidal waveform and 100% modulation index. The strength of the electric field (rms value) at the sample position was 10 V/m. A significant increase in ROS levels and DNA fragmentation was found only after exposure of the astrocytes to modulated EMF for 20 min. No evident effects were detected when shorter time intervals or continuous waves were used. The irradiation conditions allowed the exclusion of any possible thermal effect. The results show the importance of the amplitude modulation in the interaction between EMF and neocortical astrocytes (Campisi, Gulino et al. 2010).

There are studies where similar effects of modulated and CW MW were observed. Adang et al. exposed Wistar albino rats to low-level CW and pulse-amplitude modulated RF during 21 months at 970 MHz (Adang, Remacle et al. 2009). Similar effects on immune system were observed in both groups.

Significant amount of *in vivo* studies under varying parameters of exposure (intensity, frequency, exposure time, modulation, intermittence) have been performed in Russia/Soviet Union and published in Russian. Retrospective analysis of 52 Russian/Soviet *in vivo* studies with animals (mice, rats, rabbits, guinea pigs) on chronic exposure to MW has recently been published (Grigoriev, Stepanov et al. 2003). In these studies, various endpoints were measured up to 4 month of chronic exposure including analysis of: weight of animal body, histological analysis and weight of tissues, central nervous system, arterial pressure, blood and hormonal status, immune system, metabolism and enzymatic activity, reproductive system, teratogenic and genetic effects. Based on their analysis, the authors concluded that: “exposure to modulated MW resulted in bioeffects, which can be different from the bioeffects induced by CW MW; exposure to modulated MW at low intensities (non-thermal levels) could result in development of unfavorable effects; direction and amplitude of the biological response to non-thermal MW, both *in vitro* and *in vivo*, depended on type of modulation; often, but not always, modulated MW resulted in more pronounced bioeffects than CW MW; the role of modulation was more pronounced at lower intensity levels”.

One review of the Russian/Soviet studies on the role of modulation on MW effects is available in English (Pakhomov and Murphy 2000). The authors conclude that “a number of good-quality studies have convincingly demonstrated significant bioeffects of pulsed MW. Modulation often was the factor that determined the biological response to irradiation, and reactions to pulsed and CW emissions at equal time-averaged intensities in many cases were substantially different". Since that time, more studies have been published in Russian which show the role of modulation in experiemnts with animals (Dolgacheva, Semenova et al. 2000; Pashovkina and Akoev 2000; Pashovkina and Akoev 2001; Pashovkina and Akoev 2001; Akoev, Pashovkina et al. 2002).

In conclusion, significant amount of in vitro and in vivo studies from different research groups, although not universally reported, clearly indicated dependence of the NT MW effects on modulation.

IX. POLARIZATION

Polarization is a property of electromagnetic waves that describes the orientation of their oscillations versus direction of propagation. In most cases, electromagnetic wave propagates in free space as a transverse wave - the polarization is perpendicular to the wave's direction of propagation. The electric field may be oriented in a single direction (linear polarization), or it may rotate as the wave propagates (circular or elliptical polarization). In the latter cases, the oscillations can rotate either towards the right (right-handed polarization) or towards the left (left-handed polarization) in the direction of propagation.

The effects of circularly polarized (CP) MW were studied in *E. coli* cells at the frequencies from two frequency windows (resonances) that were identified using linearly polarized (LP) MW, within the frequency ranges of 51.62-51.84 GHz and 41.25-41.50 GHz (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992). At the resonance frequency of 51.76 GHz, right-handed CP MW inhibited repair of X-ray-induced DNA damages (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992). In contrast to right-handed polarization, left-handed CP MW had virtually no effect on the DNA repair, while the efficiency of LP MW was in-between of two circular polarizations. Inversion in effectiveness of circular polarizations was observed at another resonance frequency, 41.32 GHz. In contrast to the frequency of 51.76 GHz, left-handed CP MW at 41.32 GHz significantly inhibited DNA repair, while right polarization was almost ineffective. MW of the same CP affected cells at several frequencies tested within each resonance, alternative CP being almost ineffective (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992; Belyaev, Shcheglov et al. 1992). Therefore, specific sign of effective CP, either left- or right-, was the attribute of each resonance. Two different types of installations, based on either spiral waveguides (Belyaev, Shcheglov et al. 1992) or quarter-wave mica plates (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992; Shcheglov, Belyaev et al. 1997; Ushakov, Shcheglov et al. 1999; Ushakov, Alipov et al. 2005), were used to produce CP MW. Similar results were observed regardless the way of producing the MW of different polarizations.

Pre-irradiation of *E. coli* cells to X-rays inverted the sign of effective polarization (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992). This inversion was observed for two different resonances, 41.32 and 51.76 GHz. Neither resonance frequencies, nor half-widths of the resonance changed during the inversions in effective CPs. The effects of left- and right-handed CP MW become the same at 50 cGy (Belyaev, Alipov et al. 1992). At this dose, about one single stranded DNA break per haploid genome was induced. X-ray-induced DNA breaks result in relaxation of the supercoiled DNA-domains. It is known that the majority of DNA in living cells has a right-handed helicity (B-form) but a minor part, in order of 1 %, may alternate from the B-form with the form of left-handed helix (Z-form). Supercoiling is connected with transitions between right B-form to left Z-form in these DNA sequences. Therefore, the data suggested that difference in biological effects of polarized MW might be connected with DNA helicity and supercoiling of DNA-domains.

Supercoiling of DNA-domains is changed during cell cycle because of transcription, replication, repair, and recombination. It can also be changed by means of DNA-specific intercalators such as ethidium bromide (EtBr). EtBr changes supercoiling and facilitates the transition of DNA sequences from Z-form to B-form. Preincubation of *E. coli* AB1157 cells with EtBr inverted the effective polarization at the resonance frequency of 51.755 GHz and right-handed MW became more effective than left polarization (Ushakov, Shcheglov et al. 1999). EtBr changed the supercoiling of DNA-domains starting at a concentration of 1 μg/ml as measured with the AVTD in different cell types including *E. coli* (Belyaev, Shcheglov et al. 1996; Belyaev, Alipov et al. 1997; Belyaev, Eriksson et al. 1999). These data provided further evidence that DNA may be a target for the NT MW effects.

The effects of MW on conformation of nucleoids in *E. coli* cells have recently been studied at the power flux density of 100 $\mu W/cm^2$ (Ushakov, Alipov et al. 2006). Linearly polarized MW resulted in significant effects within specific frequency windows of resonance type in the range of 51-52 GHz. The distances between frequency windows were about 55-180 MHz. Only one of the two possible circular polarizations, left-handed or right-handed, was effective at each frequency window. The sign of effective circular polarization alternated between frequency windows.

While most data on the role of polarization in MW effects on chromatin have been obtained by the same research group (Belyaev, Alipov et al. 1992; Belyaev, Shcheglov et al. 1992; Belyaev, Shcheglov et al. 1992; Alipov, Belyaev et al. 1993; Belyaev, Alipov et al. 1993; Belyaev, Shcheglov et al. 1993; Belyaev and Kravchenko 1994; Shcheglov, Belyaev et al. 1997; Ushakov, Shcheglov et al. 1999; Ushakov, Alipov et al. 2005; Ushakov, Alipov et al. 2006), recent data of others corroborated our findings at least partially (Shckorbatov, Pasiuga et al. 2009). These authors analyzed the condensation of chromatin in human buccal epithelium cells and human fibroblasts by the method of vital indigo carmine staining. MW induced chromatin condensation in dependence on polarization (Shckorbatov, Pasiuga et al. 2009). The same research group investigated the effects influence of linear and left-handed and right-handed elliptically polarized MW at 36.65 GHz on chromatin in human fibroblast nuclei (Shckorbatov, Pasiuga et al. 2010). Microwave irradiation at 10 and 100 $\mu W/cm^2$ induced chromatin condensation. The right-handed elliptically polarized radiation was more active than the left-handed polarization.

Obviously, the difference in effects of right- and left polarizations could not be explained by the heating or by the mechanism dealing with "hot-spots" due to unequal SAR distribution. The data about the difference in effects of differently polarized MW, the inversion of effective circular polarization between resonances and after irradiation of cells with X-rays and incubation with EtBr provided strong evidence for the non-thermal mechanisms of MW effects. These data suggested chiral asymmetry in the target for the NT MW effects, one of which is presumably chromosomal DNA (Belyaev, Alipov et al. 1992), and selection rules on helicity if quantum-mechanical approach is applied (Belyaev, Shcheglov et al. 1992).

Lai and Singh have consistently reported that circularly polarized MW exposure at 2450 MHz induced DNA damage in brain cells of the exposed rats (Lai and Singh 1995; Lai and Singh 1996; Lai and Singh 1997). Replication studies have also tested circularly polarized MW exposure at 2450 MHz and no induced DNA damage was reported (Malyapa, Ahern et al. 1997; Malyapa, Ahern et al. 1998; Lagroye, Anane et al. 2004). All these replication studies have used another exposure system. However, handedness of circular polarization has not been described neither in original study, no in replications. If the handedness was different between studies it could reasonably account for inconsistency.

In some studies, MW of circular polarization with undefined handedness were used, but the obtained effects were not compared with alternative circular polarization or linear polarization (Bartsch, Kupper et al. 2010).

XI. ELECTROMAGNETIC ENVIRONMENT

It is very likely that background EMF might be of importance for the MW effects. This hypothesis is based on the experimental observations that SMF, ELF magnetic fields, and MW at low intensities induced similar effects in cells under specific conditions of exposure (Belyaev, Alipov et al. 1999; Belyaev, Shcheglov et al. 2000; Belyaev and Alipov 2001; Binhi, Alipov et al. 2001; Belyaev, Hillert et al. 2005). Despite very little has been achieved for mechanistic explanation of such effects, there are attempts to consider the effects of EMF in a wide frequency range in the frames of the same physical models (Chiabrera, Bianco et al. 1991; Matronchik, Alipov et al. 1996; Chiabrera, Bianco et al. 2000; Binhi 2002; Panagopoulos, Karabarbounis et al. 2002; Matronchik and Belyaev 2005; Matronchik and Belyaev 2008).

Litovitz and colleagues found that the ELF magnetic noise inhibited the effects of MW on ODC in L929 cells (Litovitz, Penafiel et al. 1997). The ODC enhancement was found to decrease exponentially as a function of the noise root mean square amplitude. With 60 Hz amplitude-modulated MW, complete inhibition was obtained with noise levels at or above 2 μT. With the DAMPS (Digital Advanced Mobile Phone System) cellular phone MW, complete inhibition occurred with noise levels at or above 5 μT. Further studies by the same group revealed that the superposition of ELF noise inhibited hypoxia de-protection caused by long term repeated exposures of chick embryos to MW (Di Carlo, White et al. 2002).

The effect of a magnetic noise on microwave-induced spatial learning deficit in the rat was investigated by Lai (Lai 2004). Rats were exposed to MW (2450 MHz CW, PD 2 mW/cm^2, average whole-body SAR 1.2 W/kg) alone or in combination with noise exposure (60 mG). Microwave-exposed rats had significant deficit in learning. Exposure to noise alone did not significantly affect the performance of the animals. However, simultaneous exposure to noise significantly attenuated the microwave-induced spatial learning deficit. The author concluded that simultaneous exposure to a temporally incoherent magnetic field blocks MW-induced spatial learning and memory deficits in the rat (Lai 2004).

Lai and Singh studied combined effects of a temporally incoherent magnetic noise (45 mG) and MW (CW 2450 MHz, PD 1 mW/cm^2, average whole-body SAR of 0.6 W/kg) in rat brain cells (Lai and Singh 2005). MW exposure induced significant DNA breakages as measured with both neutral and alkaline comet assays. Exposure to noise alone did not significantly affect cells. However, simultaneous noise exposure blocked the MW-induced effects.

Burch et al. have analyzed the relationship between cellular telephone use and excretion of the melatonin metabolite 6-hydroxymelatonin sulfate (6-OHMS) in two populations of male electric utility workers (Study 1, *n*=149; Study 2, *n*=77) (Burch, Reif et al. 2002). Participants collected urine samples and recorded cellular telephone use over 3 consecutive workdays. Personal 60-Hz magnetic field (MF) and ambient light exposures were characterized on the same days. A repeated measures analysis was used to assess the effects of cellular telephone use, alone and combined with

MF exposures, after adjustment for age, participation month and light exposure. No change in 6-OHMS excretion was observed among those with daily cellular telephone use >25 min in Study 1 (5 worker-days). Study 2 workers with >25 min cellular telephone use per day (13 worker-days) had lower creatinine-adjusted mean nocturnal 6-OHMS concentrations (p=0.05) and overnight 6-OHMS excretion (p=0.03) compared with those without cellular telephone use. There was also a linear trend of decreasing mean nocturnal 6-OHMS/creatinine concentrations (p=0.02) and overnight 6-OHMS excretion (p=0.08) across categories of increasing cellular telephone use. A combined effect of cellular telephone use and occupational 60-Hz MF exposure in reducing 6-OHMS excretion was also observed in Study 2. The authors concluded that exposure-related reductions in 6-OHMS excretion were observed in Study 2, where daily cellular telephone use of >25min was more prevalent. Prolonged use of cellular telephones may lead to reduced melatonin production, and elevated 60-Hz MF exposures may potentiate the effect.

Yao and colleagues investigated the influence of the GSM-like MW at 1.8 GHz on DNA damage and intracellular reactive oxygen species (ROS) formation in human lens epithelial cells (hLECs) (Yao, Wu et al. 2008). DNA damage examined by alkaline comet assay was significantly increased after 3 W/kg and 4 W/kg radiation, whereas the double-strand breaks (DSB) evaluated by γ-H2AX foci were significantly increased only after 4 W/kg radiation. Significantly elevated intracellular ROS levels were detected in the 3-W/kg and 4-W/kg groups. After exposure to 4 W/kg for 24 hours, hLECs exhibited significant G_0/G_1 arrest. All the effects were blocked when the MW exposure was superposed with a 2 μT electromagnetic noise. The authors concluded that superposed electromagnetic noise blocks MW-induced DNA damage, ROS formation, and cell cycle arrest.

It has previously been reported that resonance effects of MW on *E. coli* cell depend on the magnitude of static magnetic field at the place of MW exposure (Belyaev, Alipov et al. 1994). This dependence was explained by the model of electron-conformational interactions that also predicted possible shift of resonance frequencies in dependence on SMF (Belyaev, Shcheglov et al. 1996).

More recently, Ushakov with co-authors exposed *E. coli* cells to MW at the PD of 10^{-10} W/cm^2 and the frequencies of 51.675, 51.755 and 51.835 GHz (Ushakov, Alipov et al. 2005). In this study, cells were exposed to MW at various values of SMF within the range of geomagnetic filed: 22, 49, 61, or 90 μT. The authors observed that the effects of MW exposure on the conformation of nucleoids depended on the SMF during exposure.

Gapeev at al. analyzed effects of MW (41.85-42.1 GHz, frequency increment 50 MHz, PD 50 μВт/cm^2, 20 min exposure) on synergistic reaction of calcium ionophore A23187 and phorbol ester PMA in activation of the respiratory burst of the peritoneal neutrophils of mice (Gapeev, Iakushina et al. 1997). The MW exposure was performed at various SMF. At a SMF of 50 μT, the authors observed frequency-dependent inhibition of the synergetic reaction with maximal effect at the frequency of 41.95 GHz. In the same frequency range, frequency-dependent activation of the synergetic reaction with a maximal effect at the frequency of 42.0 GHz was found at a SMF of 95 μT. The authors concluded that increasing the SMF from 50 to 95 μT resulted in the inversion of ten MW effects and the shift of the resonance frequency by 50 MHz (Gapeev, Iakushina et al. 1997; Gapeev, Iakushina et al. 1999). Moreover, these effects of MW at the 41.95 GHz and 42.0 GHz were not found at the SMF of ±1, 28.3, 75.5 or 117.3 μT suggesting that the NT MMW effects may appear only at specific values of SMF (Gapeev, Iakushina et al. 1997; Gapeev, Iakushina et al. 1999).

During 1997–2008, Bartsch et al. have performed two long-term (I and II) and two life-long (III and IV) experiments analyzing the effect of chronic exposure to a low-intensity GSM-like signal (900 MHz pulsed with 217 Hz, 100 μW/cm² average power flux density, 38–80 mW/kg SAR for whole body) on health and survival of unrestrained female Sprague-Dawley rats kept under identical conditions (Bartsch, Kupper et al. 2010). Radiofrequency continued up to 37 months. In experiment I no adverse health effects of chronic RF-exposure were detectable, neither by macroscopic nor detailed microscopic pathological examinations. Also in experiment II no apparent macroscopic pathological changes due to treatment were apparent. In the course of two complete survival experiments (2002–2005; 2005–2008) median survival was significantly shortened under RF-exposure in both experiments by 9.06% (95% CI 2.7 to 15.0%) (p=0.0064); i.e by 72 days in experiment III and 77 days in experiment IV (Bartsch, Kupper et al. 2010). Based on their thorough analysis of possible reasons for variability in RF effects from year to year, the authors assumed that theses variations follow the course of solar activity within the 11-years' sunspot cycle which, according to theirs reported observations, seems to affect pineal melatonin secretion which is an integral part of endogenous defense against cancer. The activity of the sun may influence laboratory animals via changes in the geomagnetic field, which is omnipresent and perceived by specific receptors, e.g. retinal melanopsin, also involved in the light-mediated synchronization of the SCN (central circadian clock of the brain) and controlling the circadian secretion of pineal melatonin.

The observations indicating dependence of the NT MW effects on SMF and EMF stray field may be of significant interest for further development of physical theory for the NT MW effects and development of safe mobile communication.

XII. CELL-TO-CELL INTERACTION IN RESPONSE TO MICROWAVES

The effects of NT MW at the resonance frequency of 51.755 GHz on conformation of nucleoids in *E. coli* cells were analyzed with respect to cell density during exposure (Belyaev, Alipov et al. 1994). The per-cell-normalized effect of MW increased by a factor of 4.7±0.5 on average if cell density increased by one order of magnitude, from $4 \cdot 10^7$ to $4 \cdot 10^8$ cell/ml. These data suggested a co-operative nature of cell response to MW, which is based on cell-to-cell interaction during exposure. This suggestion was in line with the observed partial synchronization of cells after exposure to MW.

The co-operative nature of cell response to MW at the resonance frequency of 51.755 GHz was confirmed in further studies with *E. coli* cells (Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997; Shcheglov, Alipov et al. 2002). In addition, dependence of the per-cell-normalized effect on cell density was found for two other resonances, 51.675 GHz and 51.688 GHz. These data suggested that dependence on cell density during exposure is a general attribute of the resonance response of *E. coli* cells to NT MW. At the cell density of $4 \cdot 10^8$ cells/ml, the average intercellular distance was approximately 13 μm that is 10 times larger than the linear dimensions of *E. coli* cells (Belyaev, Alipov et al. 1994; Shcheglov, Alipov et al. 2002). Therefore, no direct physical contact seemed to be involved in the cell-to-cell interaction. Two mechanisms, biochemical and electromagnetic, were considered to account for the co-operative nature in the resonance response to weak EMF in wide frequency range including ELF, MW and ionizing radiation (Belyaev 1993; Belyaev, Alipov et al. 1994; Alipov, Shcheglov et al. 2003). The first one, biochemical, is based on release of secondary chemical messengers (ions, radicals, or molecules) by those cells, which were directly targeted. Via diffusion, these messengers can induce response in other cells. The second mechanism, electromagnetic, is based on reemission of secondary photons. According to this mechanism, reemitted photons can induce response in other cells if the intercellular distance is shorter than the length of photon absorption. The experimental data on MW effects fitted better to the electromagnetic mechanism but a combination of two mechanisms was also possible (Belyaev, Alipov et al. 1994; Shcheglov, Alipov et al. 2002). In particular, radicals with prolonged lifetimes might be involved in the observed cell-to-cell communication during response to EMF (Belyaev, Alipov et al. 1998).

The absorption length of photons with the frequencies of 10^{12}-10^{13} Hz corresponds to the intracellular distance at the cell density of $5 \cdot 10^8$ cell/ml, at which saturation in the dependences of EMF effects on cell density was observed (Belyaev, Alipov et al. 1994; Belyaev, Alipov et al. 1995; Belyaev, Alipov et al. 1998; Shcheglov, Alipov et al. 2002). Such photons may be involved in cell-to-cell communication according to the electromagnetic mechanism and in agreement with the prediction of Fröhlich that biosystems support coherent excitations within frequency range of 10^{11}-10^{12} Hz (Frohlich 1968). From this point of view, cell suspension may respond to NT MW as a whole. In this case, the number of the exposed cells should be large enough to facilitate cell-to-cell communication during the responses to MW at specific parameters of exposure such as frequency, modulation, and polarization. Interestingly, the cell density for saturation of both MW and ELF effects was about $5 \cdot 10^8$ cell/ml that is close to cell densities in soft tissues of eukaryotes (Belyaev, Alipov et al. 1998; Shcheglov, Alipov et al. 2002). Such density of cells in the tissues may be important for regulation of living systems by electromagnetic cell-to-cell communication. Cellular membranes and DNA have been considered as possible sources of coherent excitations and photons, which may be involved in electromagnetic cell-to-cell communication (Frohlich 1968; Belyaev, Shcheglov et al. 1996; Belyaev, Alipov et al. 1998).

PD dependences of the MW effect at the 51.755 GHz resonance frequency were considerably different between two cell densities, $4 \cdot 10^7$ cells/ml and $4 \cdot 10^8$ cells/ml (Belyaev, Shcheglov et al. 1996). However, the resonance frequency of 51.755 GHz did not shift with the changes in cell density. The half-width of the 51.755 GHz resonance did not depend on cell density either. Contrary to the 51.755 GHz resonance response, the half-width of the 51.675 GHz resonance depended on cell density (Shcheglov, Belyaev et al. 1997). The data suggested that intracellular interaction during the NT MW exposures at some specific frequencies might affect sub-cellular targets for NT MW. This target is presumably chromosomal DNA that is organized in the DNA-domains (Belyaev, Alipov et al. 1992; Belyaev, Alipov et al. 1993; Matronchik and Belyaev 2005).

In all studies concerning dependence of the MW effects on cell density, the cells occupied a negligible part of the exposed volume and could not change the absorption of MW even at the highest cell densities (Belyaev, Alipov et al. 1994; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997; Shcheglov, Alipov et al. 2002). Striking difference in the cell responses at various cell densities provided further evidence for non-thermal mechanism of the observed MW effects.

Significant MW effect on synchronization of *Saccharomyces carlsbergensis* yeast cells were observed by Golant and co-authors (Golant, Kuznetsov et al. 1994). Exposure to MW at 30 μW/cm^2 and 46 GHz induced synchronization as measured by cell density and bud formation. The authors assumed that MW induced cell-to-cell interaction resulting in the observed synchronization.

Possible role of intrinsic electromagnetic fields in cell-to-cell communication and mechanisms of their generation have recently been reviewed (Cifra, Fields et al. 2011).

XIII. GENETIC BACKGROUND AND CELL TYPE

Belyaev et al. have studied effects of MW on *E. coli* cells of three isogenic strains with different length of chromosomal DNA (Belyaev, Alipov et al. 1993). Bacterial chromosomal DNA in the cells of N99 wild type stain was lengthened by inserting DNA from λ and $\lambda imm^{434}bio^{10}$ phages. Two strains were obtained with increased length of chromosomal DNA, N99(λ) and N99($\lambda,\lambda imm^{434}bio^{10}$). The cells of these 3 strains were exposed to MW 10^{-10} at W/cm^2 and 10-17 frequencies within the ranges of 41.24-41.37 GHz and 51.69-51.795 GHz. The changes in chromatin conformation were analyzed before and after exposure. Clear resonance responses to MW were observed for each strain in both frequency ranges. However, each strain had its own resonance frequency, which were statistically significantly different between strains. All resonances had the same amplitude and half-width (Belyaev, Alipov et al. 1993). In each frequency band, all 3 resonances had the same effective circular polarization: right-handed in the 41.24-41.37 GHz band and left-handed within 51.69-51.795 GHz. All these data have led to conclusion that lengthening of chromosomal DNA resulted in shifting the resonance MW spectra of action. Importantly, these shifts in resonance frequencies could not be explained by the genetic activity of the inserted DNA. On the other hand, theoretical consideration based on oscillations of the DNA-domains regarding a whole nucleoid provided a good correlation between the increasing in the DNA length and the shifts in resonances (Belyaev, Alipov et al. 1993). A detailed analysis of MW effects on the cells of another *E. coli* strain, AB1157, at 10^{-10} W/cm^2 and various frequencies within 51.69-51.795 GHz, revealed the resonance frequency of 51.755±0.001 GHz (Belyaev, Shcheglov et al. 1996). This value was statistically significantly different from the resonance frequency of 51.765±0.002 in response of *E. coli* N99 cells to MW in the same frequency range (Belyaev, Shcheglov et al. 1996). It should be noted that both strains, AB1157 and N99, are considered as wild type strains. Nevertheless, these strains are different in their genotypes by several gene markers (Lukashevsky and Belyaev 1990; Belyaev, Alipov et al. 1992). These data provided evidence that cells of different origin, even being considered as wild type cells, might have different resonance responses to NT MW because of differences in their genotypes.

Stagg with colleagues exposed tissue cultures of transformed and normal rat glial cells to modulated MW (TDMA that conforms to the North American digital cellular telephone standard) at 836.55 MHz (Stagg, Thomas et al. 1997). Results from DNA synthesis assays differed for these two cell types. Sham-exposed and MW-exposed cultures of primary rat glial cells showed no significant differences for either log-phase or serum-starved condition. C6 glioma cells exposed to MW at 5.9

μW/g SAR (0.9 mW/cm^2) exhibited small (20-40 %) but significant increases in 38 % of [^{3}H]-thymidine incorporation experiments.

Repacholi with co-authors chronically exposed wild-type mice and E mu-Pim1 transgenic mice, which are moderately predisposed to develop lymphoma spontaneously, to plane-wave pulse-modulated MW at 900 MHz with a pulse repetition frequency of 217 Hz and a pulse width of 0.6 ms (Repacholi, Basten et al. 1997). Incident power densities were 2.6-13 W/m^2 and SARs were 0.008-4.2 W/kg, averaging 0.13-1.4 W/kg. The lymphoma risk was found to be significantly higher in the exposed transgenic mice. No effects were seen in the wild type mice.

Markkanen with colleagues found that MW affected the UV-induced apoptosis in *Saccharomyces cerevisiae* yeast cells KFy437 (cdc48-mutant) but did not modify apoptosis in KFy417 (wild-type) cells (Markkanen, Penttinen et al. 2004).

Czyz with colleagues exposed pluripotent embryonic stem (ES) cells of wild-type and deficient for the tumor suppressor p53 to pulse modulated GSM MW at 1.71 GHz (Czyz, Guan et al. 2004). Two dominant GSM modulation schemes (GSM-217 and GSM-Talk), which generate temporal changes between GSM-Basic (active during talking phases) and GSM-DTX (discontinuous transmission, which is active during listening phases thus simulating a typical conversation), were applied to the cells at and below the ICNIRP safety standards, 2 and 1.5 W/kg . GSM-217 MW induced a significant upregulation of mRNA levels of the heat shock protein hsp70 of p53-deficient ES cells differentiating in vitro, paralleled by a low and transient increase of c-jun, c-myc, and p21 levels in p53-deficient, but not in wild-type cells. Theses data further substantiated the notion that the genetic background determines cellular responses to GSM MW.

Nylund and Leszczynski have examined cell response to MW (900 MHz GSM-like signal, average SAR of 2.8 W/kg) using two human endothelial cell lines: EA.hy926 and EA.hy926v1 (Nylund and Leszczynski 2006). Gene expression changes were examined using cDNA Expression Arrays and protein expression changes were examined using 2-DE and PDQuest software. The same genes and proteins were differently affected by exposure in each of the cell lines.

Remondini et al. analyzed changes in gene expression in six human cell lines by gene microarrays (Remondini, Nylund et al. 2006). Cells were exposed to MW at 900 MHz GSM Basic mode, SAR 1.8-2.5 W/kg, 1 h exposure. Most cell lines responded to GSM-900 MHz, except for the CHME5 human microglial cells.

Rat1 and HeLa human cells were subjected to RF exposure at a frequency of 875 MHz with an intensity of 0.07 mW/cm2 (Friedman, Kraus et al. 2007). In Rat1 cells, phosphorylation peaked at 15 min after irradiation and returned to basal level within 30 min, whereas, in HeLa cells, peak phosphorylation was at 5 min after stimulation and decreased thereafter. Increases in Hb-

EGF release upon mobile phone irradiation were detected in both Rat1 and HeLa cell lines, although the amount released from irradiated HeLa cells was much higher than that released from Rat1 cells.

Zhao et al. studied whether expression of genes related to cell death pathways are dysregulated in primary cultured neurons and astrocytes by exposure to MW from GSM cell phone at the frequency of 1900 MHz for 2 h (Zhao, Zou et al. 2007). Microarray analysis and real-time RT-PCR have shown up-regulation of caspase-2, caspase-6 and Asc (apoptosis associated speck-like protein containing a card) gene expression in neurons and astrocytes. Up-regulation occurred in both "on" and "stand-by" modes in neurons, but only in "on" mode in astrocytes. Additionally, astrocytes showed up-regulation of the Bax gene. The authors concluded that even relatively short-term exposure to the cell phone radiation can up-regulate elements of apoptotic pathways in cells derived from the brain, and that neurons appear to be more sensitive to this effect than astrocytes.

Hoyto et al. analyzed the effects of MW exposure on cellular ornithine decarboxylase (ODC) activity in fibroblasts, two neural cell lines and primary astrocytes (Hoyto, Juutilainen et al. 2007). Several exposure times and exposure levels were used, and the fields were either unmodulated or GSM-like-modulated. Murine L929 fibroblasts, rat C6 glioblastoma cells, human SH-SY5Y neuroblastoma cells, and rat primary astrocytes were exposed to RF radiation at 872 MHz in a waveguide exposure chamber equipped with water cooling. Cells were exposed for 2, 8, or 24 hours to CW MW or to a GSM type signal pulse modulated at 217 Hz. ODC activity in rat primary astrocytes was decreased statistically significantly and consistently in all experiments performed at two exposure levels (1.5 and 6.0 W/kg) and using GSM modulated or CW radiation. In the secondary cell lines, ODC activity was generally not affected. The authors concluded that ODC activity was affected by MW exposure in rat primary neural cells, but the secondary cells used in this study showed essentially no response. In further studies by the same group, the difference in response of human SH-SY5Y neuroblastoma and mouse L929 fibroblast cells to a GSM-modulated MW at 872 MHz was replicated (Hoyto, Luukkonen et al. 2008).

Human cultured fibroblasts of three different donors and three different short-term human lymphocyte cultures were exposed to UMTS-like MW at 1950 MHz and the SAR below safety limit of 2 W/kg by Schwarz et al. (Schwarz, Kratochvil et al. 2008). The alkaline comet assay and the micronucleus assay were used to analyze genotoxic effects. UMTS exposure increased the comet tail factor (CTF) and induced centromere-negative micronuclei in human cultured fibroblasts in a dose and time-dependent way. No UMTS effect was obtained with lymphocytes, either unstimulated or stimulated with phytohemagglutinin. The authors concluded that UMTS exposure may cause genetic alterations in some but not in all human cells in vitro.

Del Vecchio et al. have tested viability, proliferation, and vulnerability of neural cells, after continuous radiofrequency (RF) electromagnetic fields exposure (global system for mobile telecommunications (GSM) modulated 900 MHz signal at a specific absorption rate (SAR) of 1 W/kg and maximum duration 144 h) generated by transverse electromagnetic cells. Two cellular systems, SN56 cholinergic cell line and rat primary cortical neurons were used (Del Vecchio, Giuliani et al. 2009). Exposure to RF did not change viability/proliferation rate of the SN56 cholinergic cells or viability of cortical neurons. Co-exposure to RF exacerbated neurotoxic effect of hydrogen peroxide in SN56, but not in primary cortical neurons, whereas no cooperative effects of RF with glutamate and 25-35AA beta-amyloid were found. These data suggest that only under particular circumstances (cell type and type of co-exposure) exposure to GSM modulated, 900MHz signal act as a co-stressor for oxidative damage of neural cells.

Gerner et al. exposed four different human cell types exposed to modulated GSM 1800 MHz at 2 W/kg (Gerner, Haudek et al. 2010). While short-term exposure did not significantly alter the proteome, an 8-h exposure caused a significant increase in protein synthesis in Jurkat T-cells and human fibroblasts, and to a lesser extent in activated primary human mononuclear cells (Gerner, Haudek et al. 2010). Quiescent (metabolically inactive) mononuclear white blood cells, did not detectably respond to GSM 1800 MHz. Most of the proteins found to be induced were chaperones, which are mediators of protein folding. Heat-induced proteome alterations detectable with used proteome methodology would require heating greater than 1°C. Because GSM-induced heating was less than 0.15°C, a heat-related response was excluded.

Dragicevic et al. evaluated brain mitochondrial function in aged Tg mice and non-transgenic (NT) littermates following 1 month of daily exposure to EMF at 918 MHz frequency, involved modulation with Gaussian minimal-shift keying (GMSK) signal, and SAR levels that varied between 0.25 and 1.05 W/kg (Dragicevic, Bradshaw et al. 2011).The cognitively-important brain areas of cerebral cortex and hippocampus in EMF-exposed mice exhibited clear increases in maximum mitochondrial respiration, while the striatum and amgydala were unaffected. For Tg mice, long-term EMF treatment induced a dramatic reduction in mitochondrial ROS levels in both cerebral cortex and hippocampus, but not in striatum or amygdala. By contrast, NT mice given EMF treatment did not show significant changes in ROS levels within any of the four brain areas analyzed. Therefore, EMF treatment reduced ROS levels selectively in Tg mice and selectively in cognitively-important brain areas.

Finally, it follows from the emerging data that MW effects are dependent on genotype and cell-type. These dependences may explain, at least partly, the discrepancies among studies from *different laboratories and demand careful selection of biological objects in designing the replication studies.*

XIV. SEX-AND AGE-RELATED DIFFERENCES

There are few studies consistently indicating that MW may exert a sex-related influence on brain activity.

Papageorgiou and co-authors investigated the sex-related influence of MW similar to that emitted by GSM900 mobile phones on brain activity (Papageorgiou, Nanou et al. 2004). Baseline EEG energy of males was greater than that of females, and exposure to MW decreased EEG energy of males and increased that of females. Memory performance was invariant to MW exposure and sex influences.

Smythe and Costall reported the effects of mobile phone exposure on short- and long-term memory in male and female subjects (Smythe and Costall 2003). The results showed that males exposed to an active phone made fewer spatial errors than those exposed to an inactive phone condition, while females were largely unaffected. These results further indicated that mobile phone exposure has functional consequences for human subjects, and these effects appear to be sex-dependent.

Nam and colleagues exposed volunteers of both sex to MW emitted by a CDMA cellular phone for half an hour (Nam, Kim et al. 2006). Physiological parameters such as systolic and diastolic blood pressures, heart rate, respiration rate, and skin resistance were simultaneously measured. All the parameters for both groups were unaffected during the exposure except for decreased skin resistance of the male subjects (Nam, Kim et al. 2006).

Güler et al. exposed infant female and male white rabbits to 1800 MHz GSM like RF signal at SAR of 1.8 W/kg for 15 min/day during 7-14 days (Guler, Tomruk et al. 2012). Lipid peroxidation levels in the liver tissues of female and male infant rabbits increased under RF radiation exposure. Liver 8-hydroxy-2 '-deoxyguanosine (8-OHdG) levels of female rabbits exposed to RF radiation were also found to increase when compared with the levels of non-exposed infants. However, there were no changes in liver 8-OHdG levels of male rabbits under RF exposure.

Santini et al. have performed a survey study on symptoms experienced during use of digital cellular phones using questionnaire of 161 students and workers in a French engineering school (Santini, Seigne et al. 2001). A significant increase in concentration difficult ($p < 0.05$) was reported by users of 1800-MHz (DCS) cellular phones compared to 900-MHz (GSM) phone users.

In users of cellular phones, women significantly ($p < 0.05$) complained more often of sleep disturbance than men. This sex difference for sleep complaint was not observed between women and men non-users of cellular phone. The use of both cellular phones and VDT significantly increased concentration difficulty. Digital cellular phone users also significantly ($p < 0.05$) more often complained of discomfort, warmth, and picking on the ear during phone conversation in relation with calling duration per day and number of calls per day. The complaint warmth on the ear might be a signal to users for stopping the call.

Prevalence of women (usually around 70%) among subjects, which report hypersensitivity to electromagnetic fields of wide frequency range including MW, may also provide indirect evidence for the gender-dependent effects of MW.

In his pioneering study concerning age in cancer risk from MW exposure, Hardell and colleagues found that the highest risks were associated with >5-year latency period in the youngest age group studied, 20-29-year, for analog phones (OR = 8.17, 95% CI = 0.94-71), and cordless phones (OR = 4.30, 95% CI = 1.22-15) (Hardell, Mild et al. 2004). Of note, no participants of age less 20 years were involved on this study. In further studies from the Hardell's group, highest risk was found in the age group <20 years at time of first use of wireless phones (Hardell and Carlberg 2009; Hardell, Carlberg et al. 2009).

Nam with co-authors reported that skin resistance in teenagers decreased by exposure to CDMA MW from cellular phones whereas no effects were seen in adults (Nam, Kim et al. 2006).

Capri et al. analyzed CD25, CD95, CD28 molecules in unstimulated and stimulated CD4+ e CD8+ T cells in vitro (Capri, Salvioli et al. 2006). Peripheral blood mononuclear cells (PBMCs) from young and elderly donors were exposed or sham-exposed to RF (1,800 MHz, SAR 2 W/kg) with or without mitogenic stimulation. No significant changes in the percentage of these cell subsets were found between exposed and sham-exposed lymphocytes in both young and elderly donors. Nevertheless, RF exposure induced a slight, but significant, downregulation of CD95 expression in stimulated CD4+ T lymphocytes from elderly, but not from young donors. This age-related result is noteworthy given the importance of such molecule in regulation of the immune response.

XV. INDIVIDUAL TRAITS

Shckorbatov et al. investigated electrokinetic properties of cell nuclei and condensation of heterochromatin in human buccal epithelium cells in response to MW at 42.2 GHz (Shckorbatov, Grigoryeva et al. 1998). MW exposure decreased electric charge of cell nuclei and an increased chromatin condensation in dependence on individual traits of donors.

Individual variability in effects of GSM and UMTS MW on chromatin conformation and 53BP1/γ-H2AX DNA repair foci was observed in studies with lymphocytes from hypersensitive to EMF subjects and healthy persons (Sarimov, Malmgren et al. 2004; Belyaev, Hillert et al. 2005; Markova, Hillert et al. 2005; Belyaev, Markova et al. 2009). The same individual variability was reported for response of chromatin condensation human lymphocytes to ELF magnetic fields (Sarimov, Alipov et al. 2011). This variability correlated with initial state of chromatin in the exposed cells (Sarimov, Alipov et al. 2011). Thus, the data from two different research groups have indicated that the NT MW effects on human cells depended on initial sate of chromatin that individually varied between subjects.

Zotti-Martelli with colleagues exposed peripheral blood lymphocytes from nine different healthy donors for 60, 120 and 180 min to CW MW with a frequency of 1800 MHz and PD of 5, 10, and 20 mW/cm^2 and analyzed DNA damage using micronucleus (MN) assay (Zotti-Martelli, Peccatori et al. 2005). Both spontaneous and induced MN frequencies varied in a highly significant way among donors, and a statistically significant increase of MN, although rather low, was observed dependent on exposure time and PD. The data analysis highlighted a wide inter-individual and reproducible variability in the response.

Hinrikus et al. (Hinrikus, Bachmann et al. 2008) evaluated the effects of pulse-modulated MW (450 MHz) on human EEG rhythms. Thirteen healthy volunteers were exposed to MW; the field power density at the scalp was 0.16 m W/cm^2. Differences were found in individual sensitivity to exposure. Increases in the EEG beta power appeared statistically significant in the case of four subjects. In other study, the same authors confirmed and extended their observations on individual sensitivity to exposure with pulse-modulated MW. The experiments were carried out on four different groups of healthy volunteers. A 450-MHz MW modulated at 7 Hz (first group), 14 and 21 Hz (second group), 40 and 70 Hz (third group), 217 and 1000 Hz (fourth group) frequencies was applied. MW exposure, SAR 0.303 W/kg, increased the EEG energy. The proportion of subjects significantly affected was similar in all groups except for the 1000 Hz group: in the first group 16% at 7 Hz modulation; in the second group 31% at 14 Hz modulation and 23% at 21 Hz modulation; in the third group 20% at 40 Hz and 13% at 70 Hz modulation; in the fourth group 16% at 217 Hz and 0% at 1000 Hz modulation frequency.

Sannino et al. evaluated the induction of micronuclei in response to MW (900 MHz, average SAR of 1.25 W/kg) exposure and subsequent treatment with mitomycin C in peripheral blood lymphocytes from five human volunteers (Sannino, Sarti et al. 2009). MW exposure reduced the level of mitomycin C –induced micronuclei in cells collected from four donors (i.e., responders). However, the effect of MW was not observed in the remaining donor (i.e., non-responder). The overall data indicated the existence of heterogeneity in the MW response among individuals.

Human sensitivity to radio frequency (RF) standing waves was tested using a movable reflecting wall (Huttunen, Hanninen et al. 2009). When the reflector was moved, the position of the maximums of the standing waves changed and the electromagnetic intensity changed in the body of the standing test subject. The computer with an AD-converter registered the signals of the hand movement transducer and the RF-meter with 100MHz dipole antennas. A total of 29 adults of different ages were tested. There were 9 persons whose hand movement graphs included features like the RF-meter. Six showed responses that did not correlate with the RF-meter. There were also 14 persons who did not react at all. Sensitive persons seem to react to crossing standing waves of the RF signals.

To conclude, while only few studies were performed, to evaluate individual sensitivity, the obtained results indicate dependence of response to MW exposure on individual traits.

XVI. PHYSIOLOGICAL VARIABLES: STAGE OF CELL GROWTH, TEMPERATURE, OXYGEN, DIVALENT METALS

The importance of physiological variables, which may include all conditions of cell culture growth such as aeration, the composition of the growth and exposure media, on NT MW effects has previously been reviewed (Grundler, Jentzsch et al. 1988). Since that time, significant body of new data has been accumulated unequivocally supporting the role of physiological variables for the NT MW effects, which should be carefully taken into account when replicating the original studies.

Belyaev et al. have reported that both value and direction of the MW effects strongly depended on the phase of culture growth, at which *E. coli* cells were exposed to CP or LP MW (100 $\mu W/cm^2$) at the resonance frequencies of 41.32 GHz and 51.76 GHz (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994). At logarithmic phase of growth, MW resulted in condensation of nucleoids. In contrast, MW exposure decondensed nucleoids in cells if exposure was performed at the stationary phase of growth. It is known, that the state of nucleoid condensation depends on cell activity. In stationary cells nucleoids are more condensed compared to logarithmic cells that divide actively. It was concluded that MW are able to either stimulate or inhibit activity of the cells in dependence on stage of growth, stationary or logarithmic, respectively. Higher variability in effects was observed for logarithmic phase and effects were more stable for the stationary phase that is characterized by partial synchronization of cells (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994). There was no effect at all if cells were exposed at the end of the logarithmic phase where the MW effects changed their direction from inhibition to stimulation (Belyaev, Alipov et al. 1994). Another peculiarity was observed at the very beginning of the logarithmic stage, where the condensation of chromatin induced by MW was relatively weak. The AVTD data were confirmed by the electrophoretic analysis of proteins bound to DNA (Belyaev, Shcheglov et al. 1993). The effect in the stationary phase was characterized by a decrease in the quantity of several DNA-bound proteins with molecular weights of 61, 59, 56, 26, and 15 kDa. In contrast, abundance of some DNA-bound proteins, 61, 56, 51 and 43 kDa increased after exposure at the logarithmic phase. The decrease or increase in the abundance of DNA-bound proteins correlated with the observed changes in the state of nucleoids, decondensation or condensation, respectively.

Shcheglov et al. have studied effects of MW at the PD range of 10^{-18} to $3 \cdot 10^{-3}$ W/cm^2 stationary on logarithmic and stationary cells at various cell densities (Shcheglov, Alipov et al. 2002). Relatively weak response to MW was observed in exponentially growing cells. Partially synchronized stationary cells were more sensitive, especially at the cell densities above 10^8 cell/ml. The data suggested that the co-operative responses of cells to MW vary in dependence on phase of growth.

Recent data by Ushakov and colleagues indicated that the MW effects on *E. coli* cells depended on concentration of oxygen in the cell suspension during exposure (Ushakov, Alipov et al. 2005). This dependence might suggest that oxygen concentration should be indicated in order to improve reproducibility in replication studies.

Biological systems have been shown to be very sensitive to perturbations at conditions where critical components are at phase transition points, governed by local temperature, ionic strength and pH. This phenomenon was demonstrated by independent laboratories using 2.45-GHz MW radiation associated with a phase transition in lipid-protein complexes around 20-25 ^{0}C (Olcerst, Belman et al. 1980; Fisher, Poznansky et al. 1982; Liburdy and Vanek 1985; Allis and Sinha-Robinson 1987; Liburdy and Vanek 1987).

Fisher et al. have reported an effect of low-level 2450-MHz MW on total and ouabain-sensitive 24Na$^+$ flux from human erythrocytes. Erythrocytes washed and loaded with 24Na$^+$ were exposed at an absorption rate of 2.0-3.0 mW/ml suspension in a waveguide system under temperature- controlled conditions for 1 or 2 hr. Experiments were run in parallel, with exposed and sham- irradiated (control) samples, at various temperatures between 7 and 35^{0}C. Continuous-wave electromagnetic radiation at 2450 MHz had a significant effect on 24Na$^+$ efflux, but only in the temperature range 22-25^{0}C. Total efflux increased an average of 23%; this was the result of an increase in the ouabain-insensitive component (mean, 33%) and a decrease in the ouabain- sensitive portion (mean, 18%). These results indicated increased passive Na+ efflux and decreased ATPase-mediated Na+ efflux in erythrocytes exposed to low-level microwaves at 22-25^{0}C (Fisher, Poznansky et al. 1982).

Liburdy and Vanek have shown that MW-induced protein shedding is oxygen and temperature dependent (Liburdy and Vanek 1987). Microwaves (2450 MHz, 60 mW/g) resulted in the release or shedding of at least 11 low-molecular-weight proteins (<31,000 Da) from rabbit erythrocytes maintained in physiological buffer. This release was oxygen dependent and occured in 30 min for exposures conducted within the special temperature region of 17-21^{0}C, which is linked to a structural or conformational transition in the cell membrane. Shedding of 26,000 and 24,000 Da proteins was unique to MW treatment, with enhanced release of 28,000 and < 15,000 Da species upon MW exposure. Two-dimensional isoelectric focusing revealed that proteins of< 14,000 Da shed during microwave treatment exhibited a pI of 6.8-7.3 not seen in sham-treated cells. When erythrocytes were maintained at 17-21^{0}C in the absence of divalent cations, release of 28,000-31,000 and < 14,000 Da components was detected. This indicated that cation-bridge stability may be important for release of these proteins. The results provided evidence that MW alter erythrocyte protein composition at temperatures linked to a transition in the cell membrane and that destabilization of salt bridges may play a role in an interaction mechanism for protein release (Liburdy and Vanek 1987).

The ATPase activity in human red blood cell membranes was investigated in vitro as a function of temperature and exposure to 2,450-MHz continuous wave microwave radiation to confirm and extend a report of Na^+ transport inhibition under certain conditions of temperature and exposure (Allis and Sinha-Robinson 1987). Assays were conducted spectrophotometrically during microwave exposure with a custom-made spectrophotometer-waveguide apparatus. Temperature profiles of total ATPase and Ca^{+2} ATPase (ouabain-inhibited) activity between 17 and 31 degrees C were graphed as an Arrhenius plot. Each data set was fitted to two straight lines which intersect between 23 and 24 degrees C. The difference between the total and Ca^{+2} ATPase activities, which represented the Na^+/K^+ ATPase activity, was also plotted and treated similarly to yield an intersection near 25 degrees C. Exposure of membrane suspensions to electromagnetic radiation, at a dose rate of 6 W/kg and at five temperatures between 23 and 27 degrees C, resulted in an activity change only for the Na^+/K^+ ATPase at 25 degrees C. The activity decreased by approximately 35% compared to sham-irradiated samples. A possible explanation for the unusual temperature/microwave interaction was proposed (Allis and Sinha-Robinson 1987).

Therefore, temperature may be an important variable, which should be taken into account while analyzing response of cells to MW.

Similar to the effects of ELF (Belyaev, Alipov et al. 1999), the MW effects were reported to be dependent on concentration of divalent ions (Gapeev, Iakushina et al. 1997).

In conclusion, physiological parameters such as stage of cell growth, temperature, oxygen an divalent ions temperature may be an important variable, which should be taken into account while analyzing response of cells to MW.

XVII. ANTIOXIDANTS AND RADICAL SCAVENGERS

Oxidative stress caused by biological, chemical and physical factors has been associated with increased risk of human cancer at various sites. Human cells induce and/or activate several oxidant generating enzymes that produce high concentrations of diverse free radicals and oxidants. These reactive species can damage DNA, RNA, lipids and proteins, leading to increased mutations and altered function of enzymes and proteins, thus contributing to the multistage carcinogenesis process. Control of oxidative stress is being explored as an approach to chemoprevention of human cancers (IARC 2002).

It is well known that endogenous (intracellular) free radicals, which are collectively called reactive oxygen species (ROS), arise from mitochondrial oxidative metabolism and other reactions in cells (Pollycove and Feinendegen 2003). The estimated average generation rate is $\sim 10^9$ ROS per cell per day (Beckman and Ames 1998), which results in 10^6 oxidative DNA damage, 10^5 SSBs and 0.1 DSBs per cell per day (Pollycove and Feinendegen 2003).

In their pioneering study, Lai and Singh described the effects of MW on the rat brain cells as measured using a microgel electrophoresis assay (Lai and Singh 1996). These effects were significantly blocked by treatment of rats either with the spin-trap compound N-tert-butyl-α-phenylnitrone or with melatonin, both agents being free radical scavengers and antioxidants (Lai and Singh 1997). These data suggested that free radicals might be involved in the effects of MW. The ability of scavengers and antioxidants has been tested by many other research groups an in all cases, this treatment inhibiter the reported TN MW effects.

Oktem andcolleagues exposed rats to MW from GSM900 mobile phone with and without melatonin treatment (Oktem, Ozguner et al. 2005). Malondialdehyde (MDA), an index of lipid peroxidation, and urine N-acetyl-beta-d-glucosaminidase (NAG), a marker of renal tubular damage, were used as markers of oxidative stress-induced renal impairment. Superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px) activities were studied to evaluate changes in antioxidant status. In the MW-exposed group, while tissue MDA and urine NAG levels increased, SOD, CAT, and GSH-Px activities were reduced. Melatonin treatment inhibited these effects. The authors concluded that melatonin might exhibit a protective effect on mobile phone-induced renal impairment in rats.

Ozguner and colleagues exposed Wistar-Albino rats to MW from GSM900 mobile phone with and without melatonin and analyzed histopathologic changes in skin (Ozguner, Aydin et al. 2004). MW induced increase in thickness of stratum corneum, atrophy of epidermis, papillamatosis, basal cell proliferation, granular cell layer (hypergranulosis) in epidermis and capillary proliferation. Impairment in collagen tissue distribution and separation of collagen bundles in dermis were all observed in exposed animals as compared to the control group. Most of these changes, except hypergranulosis, were prevented with melatonin treatment. The authors concluded that exposure to GSM900 MW caused mild skin changes and melatonin treatment could reduce these changes. In other studies of the same group, the ability of melatonin to reduce various MW-induced effects was confirmed and inhibitory potential of the antioxidant caffeic acid phenethyl ester (CAPE) was reported (Ozguner, Altinbas et al. 2005; Ozguner, Oktem et al. 2005; Ozguner, Oktem et al. 2005; Ozguner, Bardak et al. 2006).

Ayata et al. analyzed the effects of 900 MHz MW with and without melatonin on fibrosis, lipid peroxidation, and anti-oxidant enzymes in rat skin (Ayata, Mollaoglu et al. 2004). The levels of MDA and hydroxypyroline and the activities of SOD, GSH-Px, and CAT were studied. MDA and hydroxyproline levels and activities of CAT and GSH-Px were increased significantly in the exposed group without melatonin and decreased significantly in the exposed group with melatonin. SOD activity was decreased significantly in the exposed group and this decrease was not prevented by the melatonin treatment. The authors assumed that the rats irradiated with MW suffer from increased fibrosis and lipid peroxidation and that melatonin can reduce the fibrosis and lipid peroxidation caused by MW.

Ilhan with co-authors investigated oxidative damage in brain tissue of rats exposed to GSM900 MW with and without pretreatment with Ginkgo biloba (Gb) (Ilhan, Gurel et al. 2004). MW induced oxidative damage measured as: (i) increase in MDA and nitric oxide (NO) levels in brain tissue, (ii) decrease in brain SOD and GSH-Px activities, and (iii) increase in brain xanthine oxidase and adenosine deaminase activities. These MW effects were prevented by the Gb treatment. Furthermore, Gb prevented the MW-induced cellular injury in brain tissue revealed histopathologically. The authors concluded that reactive oxygen species may play a role in the adverse effects of GSM900 MW and Gb prevents the MW-induced oxidative stress by affecting antioxidant enzymes activity in brain tissue.

Guney et al.examined 900 MHz mobile phone-induced oxidative stress that promotes production of ROS and investigated the role of vitamins E and C, which have antioxidant properties, on endometrial tissue against possible 900 MHz mobile phone-induced endometrial impairment in rats (Guney, Ozguner et al. 2007). The animals were randomly grouped (eight each) as follows: 1) Control group (without stress and EMR, Group I), 2) sham-operated rats stayed without exposure to EMR (exposure device off, Group II), 3) rats exposed to 900 MHz EMR (EMR group, Group III) and 4) a 900 MHz EMR exposed + vitamin-treated group (EMR + Vit group, Group IV). A 900 MHz EMR was applied to EMR and EMR + Vit group 30 min/day, for 30 days. Endometrial levels of nitric oxide (NO, an oxidant product) and malondialdehyde (MDA, an index of lipid peroxidation), increased in EMR exposed rats while the combined vitamins E and C caused a significant reduction in the levels of NO and MDA. Likewise, endometrial superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) activities decreased in EMR exposed animals while vitamins E and C caused a significant increase in the activities of these antioxidant enzymes. In the EMR group histopathologic changes in endometrium, diffuse and severe apoptosis was present in the endometrial surface epithelial and glandular cells and the stromal cells. Diffuse eosinophilic leucocyte and lymphocyte infiltration were observed in the endometrial stroma whereas the combination of vitamins E and C caused a significant decrease in these effects of EMR. It is concluded that oxidative endometrial damage plays an important role in the 900 MHz mobile phone-induced endometrial impairment and the modulation of oxidative stress with vitamins E and C reduces the 900 MHz mobile phone-induced endometrial damage both at biochemical and histological levels.

Koylu et al. studied the effects of MW on the brain lipid peroxidation in rats, and the possible protective effects of melatonin on brain degeneration induced by MW (Koylu, Mollaoglu et al. 2006). The levels of lipid peroxidation in the brain cortex and hippocampus increased in the MW group compared with the control group, although the levels in the hippocampus were decreased by combined administration of MW and melatonin. Brain cortex lipid peroxidation levels were unaffected by melatonin treatment. The authors concluded that melatonin may prevent MW-induced oxidative stress in the hippocampus by strengthening the antioxidant defense system.

Balci et all exposed albino Wistar rats to mobile-phone-emitted radiation and analyzed oxidant/antioxidant balance in corneal and lens tissues. The results of this study suggest that mobile telephone radiation leads to oxidative stress in corneal and lens tissues and that antioxidants such as vitamin C can help to prevent these effects (Balci, Devrim et al. 2007).

Sokolovic et al. evaluated the intensity of oxidative stress in the brain of Wistar rats chronically exposed to MW from mobile phones (SAR = 0.043-0.135 W/kg) during 20, 40 and 60 days (Sokolovic, Djindjic et al. 2008). A significant increase in brain tissue malondialdehyde (MDA) and carbonyl group concentration was found. Decreased activity of catalase (CAT) and increased activity of xanthine oxidase (XO) remained after 40 and 60 days of MW exposure. Melatonin treatment significantly prevented the increases in MDA content and XO activity in the brain tissue after 40 days of exposure while it was unable to prevent the decrease of CAT activity and increase of carbonyl group contents. The authors concluded that exposure to the mobile phone MW caused oxidative damage in the brain and that treatment with melatonin significantly prevented this oxidative damage.

Gajski and Garaj-Vrhovac investigated the radioprotective effect of bee venom against DNA damage induced by 915-MHz microwave radiation (SAR of 0.6 W/kg) (Gajski and Garaj-Vrhovac 2009). Whole blood lymphocytes of Wistar rats are treated with 1 mg/mL bee venom 4 hours prior to and immediately before irradiation. Standard and formamidopyrimidine-DNA glycosylase (Fpg)–modified comet assays were used to assess basal and oxidative DNA damage produced by ROS. Bee venom decreased basal and oxidative DNA damage induced by microwave radiation. The difference between the comet assay results in the presence and in the absence of Fpg-enzyme suggested that oxidative stress is responsible for the DNA damage induced by microwave radiation. Among other possible mechanisms, antioxidant activity of bee venom may likely account for the radioporotective effect.

Esmekaya et al. analyzed effects of 1.8 GHz GSM alone and in combination with Ginkgo biloba (EGb 761) pre-treatment in human peripheral blood lymphocytes (Esmekaya, Aytekin et al. 2011). RF exposure significantly increased frequency of sister chromatid exchanges (SCE) and inhibited cell viability. No temperature difference was observed between sham control and RF exposed cells, so the observed effects may be considered as non-thermal. EGb 761 pre-treatment significantly reduced both RF effects. The authors concluded that EGb 761 had a protective role against RF induced mutagenesis.

Ozgur et al investigated oxidative damage and antioxidant enzyme status in the liver of guinea pigs exposed to mobile phone-like radiofrequency radiation (RFR) and the potential protective effects of N-acetyl cysteine (NAC) and epigallocatechin-gallate (EGCG) on the oxidative damage (Ozgur, Gler et al. 2010). Nine groups of guinea pigs were used to study the effects of exposure to an 1800-MHz Global System for Mobile Communications (GSM)-modulated signal (average whole body Specific Absorption Rate (SAR) of 0.38W/kg, 10 or 20 min per day for seven days) and treatment with antioxidants. Significant increases in malondialdehyde (MDA) and total nitric oxide (NO) levels and decreases in activities of superoxide dismutase (SOD), myeloperoxidase (MPO) and glutathione peroxidase (GSH-Px) were observed in the liver of guinea pigs after RFR exposure. NAC treatment induced increase in hepatic GSH-Px activities, whereas EGCG treatment alone attenuated MDA level. Extent of oxidative damage was found to be proportional to the duration of exposure. Authors concluded that the adverse effect of RFR may be related to the duration of mobile phone use. NAC and EGCG may protect the liver tissue against the RFR-induced oxidative damage and enhance antioxidant enzyme activities.

Female rats were exposed to a mobile phone signal (900 MHz), the mobile phone plus vitamin C group was exposed to a mobile phone signal (900 MHz) and treated orally with vitamin C (Imge, Kilicoglu et al. 2010). Malondialdehyde (MDA), antioxidant potential (AOP), superoxide dismutase, catalase (CAT), glutathione peroxidase (GSH-Px), xanthine oxidase, adenosine deaminase (ADA) and 5'nucleotidase (5'-NT) were analyzed in brain tissues. MW exposure caused an inhibition in 5'-NT and CAT activities. GSH-Px activity and the MDA level were also found to be reduced in the mobile phone group but not significantly. Vitamin C caused a significant increase in the activity of GSH-Px and non-significant increase in the activities of 5'-NT, ADA and CAT enzymes. The results suggest that vitamin C may play a protective role against detrimental effects of mobile phone radiation in brain tissue.

To conclude this section, several studies consistently show that supplementation with antioxidants and radical scavengers can reduce MW effects. In other words, the level of radicals should be considered as an important parameter for the NT MW effects. Moreover, these studies indicate that induction of radicals is one of the key events in bioeffeds of NT MW.

XVIII. CO-EXPOSURE

Zmyslony et al have studied effects of 930 MHz continuous wave (CW) electromagnetic field, 1.5 W/kg, on the reactive oxygen species (ROS) level in rat lymphocytes (Zmyslony, Politanski et al. 2004). Acute (5 and 15 min) exposure did not induce ROS. However, this exposure increased effect of $FeCl_2$, 10 µg/ml.

Co-exposure to RF (global system for mobile telecommunications (GSM) modulated 900MHz signal at a specific absorption rate (SAR) of 1 W/kg and maximum duration 144 h) exacerbated neurotoxic effect of hydrogen peroxide in SN56, but not in primary cortical neurons (Del Vecchio, Giuliani et al. 2009). These data suggest that only under particular circumstances (cell type and type of co-exposure) exposure to GSM modulated, 900MHz signal act as a co-stressor for oxidative damage of neural cells.

XIX. REPLICATION STUDIES

Obviously, not taking into account the dependences of NT MW effects on a number of physical parameters and biological variables may result in misleading conclusions regarding the reproducibility of these effects. Especially important might be the observations that NT MW could inhibit or stimulate the same functions dependent on conditions of exposure (Pakhomov, Akyel et al. 1998). Under different conditions of exposure, MW either increased or decreased the growth rate of yeast cells (Grundler, Jentzsch et al. 1988), the radiation-induced damages in mice (Sevast'yanova 1981), the respiratory burst in neutrophils of mice (Gapeev, Iakushina et al. 1997), the condensation of nucleoids in *E coli* cells (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994) and human lymphocytes (Sarimov, Malmgren et al. 2004). Potentially bi-directional effects of MW should be taken into account in replication studies.

In some cases when the conditions were kept in strict control, the effects we reproduced. Highly resonant effects of ultra-weak MW (near 70 GHz) on the induction of λ-phage were first established by Webb (Webb 1979), and subsequently corroborated (Lukashevsky and Belyaev 1990).

Despite of considerable body of studies with NT MW in biology, only a few studies were performed to independently replicate the original data on the NT MW effects. It should be noted, that these replications are usually not completely comparable with the original studies because of either missing description of important parameters of exposure or significant differences in these parameters between original study and replication. One well-known attempt to replicate the results of Gründler was the study by Gos and co-authors (Gos, Eicher et al. 1997). No MW effects were observed in this replication study. However, the deviations from the Gründler’s protocol might be a simple reason for poor reproducibility. For example, synchronized cells were used in studies of Gründler. Contrary to the Gründler’s original protocol, Gos used exponentially growing cells. If the MW effects in yeast cells are dependent on stage of growth, cell density and intercellular interactions as it has been described for *E. coli* cells (Belyaev, Shcheglov et al. 1993; Belyaev, Alipov et al. 1994; Belyaev, Shcheglov et al. 1996; Shcheglov, Belyaev et al. 1997), no response should be expected in the logarithmic phase of growth. Gos and colleagues used *S. cerevisiae* strain with the auxotrophy mutations for leucine and uracil. Gründler used the wild type strain. It might suggest another cause for the deviations between the data of Gründler and Gos. Despite orientation of SMF in respect to electric and magnetic components of MW was the same, the values of SMF were different. The stray ELF field was 120 nT in the study by Gos, that is higher than usually observed background fields, < 50 nT. The spectral characteristics of the background fields, which were described only in the study by Gos, might be also different. In addition, the conditions of cell cultivation might vary between studies; for example, the data on oxygen concentration in media used in both studies are not available.

Lai and Singh have consistently reported that circularly polarized MW exposure at 2450 MHz induced DNA damage in brain cells of the exposed rats (Lai and Singh 1995; Lai and Singh 1996; Lai and Singh 1997). Replication studies have also tested circularly polarized MW exposure at 2450 MHz and no induced DNA damage was reported (Malyapa, Ahern et al. 1997; Malyapa, Ahern et al. 1998; Lagroye, Anane et al. 2004). All these replication studies have used another exposure system. However, handedness of circular polarization has not been given neither in original study, no in replications. If the handedness was different between studies it could reasonably account for inconsistency.

Most reviews of the experimental studies do not include analysis of various biological variables and physical parameters when comparing the data on the NT MW effects from different studies. As result, misleading conclusion is often made that MW at NT levels produce no "reproducible" effects.

XX. SIMILARITY OF MICROWAVE AND ELF EFFECTS

Mobile phones not only expose the user to RF EMF but also to ELF EMF (Linde and Mild 1997; Heath, Jenvey et al. 1998; Jokela, Puranen et al. 2004; Ilvonen, Sihvonen et al. 2005; Cook, Saucier et al. 2006; Perentos, Iskra et al. 2007). Perentos et al. have recently measured and characterized the ELF magnetic field from several commercial GSM handsets (the RF characteristics being already well understood) using different probes which covered frequency range from static magnetic fields ("0 Hz") to 2 GHz. Peak ELF fields at the front sides of 5 commercial GSM phones were assessed and a maximum of 22.4 μT was reported (Perentos, Iskra et al. 2008). The main ELF component at the 217 Hz was about 1 μT at the distance of 3 cm from the handset front side. The overall pulse peak was 4.2 times greater than the 217 Hz component. 217 Hz magnetic field decreased with distance and reached 0.3 μT approximately at 5 cm from the front handset side. The overall ELF pulse peak produced by all ELF components was 4.2 times greater than the 217 Hz component. The ELF fields higher 0.3 μT have consistently been shown to correlate with increased risk of children leukemia in several studies covering European countries, USA and Japan (Kabuto, Nitta et al. 2006; Yang, Jin et al. 2008). Similar to RF, ELF has been classified by the IARC as possible carcinogen "2B". It has been known for long time that weak ELF fields and NT MW result to similar effects with significant overplaying of molecular biological pathways for their appearance (Adey 1981; Blank and Goodman 2009; Davanipour and Sobel 2009). Multiple data on ELF biological effects at intensities below the ICNIRP standards are available showing their complex dependence of the ELF effects on biological and physical variables (Belyaev, Alipov et al. 1999; Blank and Goodman 2009; Phillips, Singh et al. 2009; Sarimov, Alipov et al. 2011). In particular, stress response, molecular pathways for generation of reactive oxygen species (ROS), increased sensitivity of stem cells, and inhibition of melatonin production (Burch, Reif et al. 2000) were suggested as mechanisms which link observed increase in cancer risks and effects of exposure at the cellular level. EMF effects in a wide frequency range from ELF to MW have been considered in the frames of the same physical models (Chiabrera, Bianco et al. 1991; Matronchik, Alipov et al. 1996; Chiabrera, Bianco et al. 2000; Binhi 2002; Panagopoulos, Karabarbounis et al. 2002; Matronchik and Belyaev 2005; Matronchik and Belyaev 2008).

In many cases, because of ELF modulation and additional ELF fields created by the MW sources, for example by mobile phones, it is difficult to distinguish the effects of exposures to ELF and MW. Therefore, these combined exposures and their possible cancer risks should be considered in combination.

XXI. CANCER RISK ASSESSMENT FROM MECHANISTIC POINT OF VIEW

At present, a new situation has arisen when a significant part of the general population is exposed chronically (much longer than previously investigated durations of exposures) to NT MW from different types of mobile communication including GSM and UMTS/3G phones and base stations, WLAN (Wireless Local Area Networks), WPAN (Wireless Personal Area Networks such as Bluetooth), DECT (Digital Enhanced (former European) Cordless Telecommunications) wireless phones (Joseph, Frei et al. 2010). Multiple sources of mobile communication result in chronic exposure of general population to MW at the non-thermal levels. These exposures are characterized by low intensities, varieties and complexities of signals, and long-term durations of exposure that are comparable with a lifespan.

Most of the real signals that are in use in mobile communication have not been tested so far. Very little research has been done with real signals and for durations and intermittences of exposure that are relevant to chronic exposures from mobile communication. In some studies, so-called “mobile communication-like” signals were investigated that in fact were different from the real exposures in such important aspects as intensity, carrier frequency, modulation, polarization, duration and intermittence.

Emerging evidence suggests that the SAR concept, which has been widely adopted for safety standards, is not useful alone for the evaluation of health risks from NT MW of mobile communication. The role of other exposure parameters such as frequency, modulation, polarization, duration, and intermittence of exposure should be taken into account.

IARC has recently classified RF as a ‘Possible Human Carcinogen’ (Class 2B) (Baan, Grosse et al. 2011). Contrary to other panels, such as ICNIRP, whose members dismiss the NT MW effects based on their "non-reproducibility" and lack of comprehensive mechanisms, the IARC working group included scientists, which argued for existence of non-thermal effects and their complex dependence on variety of biological and physical parameters which should be included in consideration. By its classification, IARC has justified implementation of the Precautionary Principle, confirmed the existence of non-thermal effects that can cause health risks, and indicated that the current safety standards are insufficient to protect health.

The data about the effects of MW at super low intensities and significant role of duration of exposure in these effects along with the data showing that adverse effects of NT MW from GSM/UMTS mobile phones depend on carrier frequency and type of the MW signal suggest that MW from base-stations/masts, wireless routers, WI-FI and other wireless devices and exposures in common use today can also produce adverse effects at prolonged durations of exposure.

So far, most laboratory and epidemiological studies did not control important features of the NT MW effects and therefore, only limited conclusion regarding health effects of MW from mobile communication can be drawn from these studies. The group of Hardell was the first epidemiologic studying separately the MW signals from cordless phones, analogue phones and digital phones (Hardell, Hansson Mild et al. 2001; Hardell, Hansson Mild et al. 2003; Hardell, Eriksson et al. 2005; Hardell and Hansson Mild 2005). This approach is valid from the mechanistic point of view.

Nowadays, it is almost impossible to select control unexposed groups because the whole population in many countries is exposed to wide range of MW signals from various sources such as mobile phones, base stations/masts, WLAN, WPAN, DECT wireless phones and given that duration of exposure (at least 10 years for cancer latency period) is also important for the effects of NT MW along PD/SAR. Exposure from downlink sources (base stations *etc.*) may contribute up to

90% of total environmental outdoor-urban exposure in European countries while exposure to DECT phone is comparable to exposure to mobile phones (Frei, Mohler et al. 2009; Frei, Mohler et al. 2010; Joseph, Frei et al. 2010). In other words, there are no unexposed control groups available for epidemiologic studies in the developed countries. Substantial variation in relative ratio of downlink and uplink signals between countries (Joseph, Frei et al. 2010) can at least partially account for differences in epidemiologic data because of variation in exposure of control groups to downlink signals.

While several national registers (Norway, Australia, Finland, Denmark) report increased incidence of brain cancer, US and Swedish ones do not. This inconsistence may be accounted by deficit in reporting of tumors to the Swedish Cancer Registry (Hardell and Carlberg 2009).

Importantly, because the signals are completely replaced by other signals faster then once per 10 years, duration comparable with latent period, epidemiologic studies can not provide basement for assessment of upcoming new signals.

As far as different types of MW signals (carrier frequency, modulation, polarization, far and near field, intermittence, coherence, *etc.*) may produce different effects, cancer risks should ideally be estimated for each MW signal separately. In other words, one type of MW signal would correspond to one chemical compound. That means, for example, that each from 124 signals involved in GSM uplink mobile communication should be separately evaluated to fit situation accepted for estimation of cancer risks from chemical compounds.

It now appears that most, if not all, adult tissues and organs including blood and brain contain stem cells (Metcalfe and Ferguson 2008). Almost all hematopoietic and solid neoplasms arise from cancer stem cells that are dysfunctional versions of a normal stem cells. Current models for radiation carcinogenesis have paid much attention to the stochastic process of energy deposition in cells, but accumulating evidences have shown that the nature of the target cells, i.e. tissue stem cells and progenitor cells, needs to be taken into consideration (Niwa 2010; Richardson 2011). Stem cell self-renewal and progenitor differentiation is regulated by the specialized microenvironment—or "niche"—in which these cells reside (Alvarez-Buylla and Lim 2004) and which regulate stem cells (Morrison and Spradling 2008; Johansson, Cappello et al. 2010; Kim and Shivdasani 2012; Sugiyama and Nagasawa 2012)..Importance of stem cells for carcinogenesis, challenges the definition of volume for SAR determination in safety standards. Instead of random distribution of targets for carcinogenesis, localized distribution of SAR in stem cells and niches is needed. Because very small size of the niches in different tissues including the brain (Kazanis 2012), the SAR averaging should be performed at volumes much less then currently accepted 10 g. Decreasing the sensitive volume to the stem cell niches with sizes down to 10 μm (Richardson 2011) may likely put almost all mobile phones out of the current safety standards, even given that they are only based on thermal effects and do not consider any other parameters except for SAR. From point view of stem cell organization, the volume of SAR determination may be especially important for setting the safety standards for children. During brain development, most stem cells and their niches are spatially ephemeral and temporally transient as the cellular and molecular "puzzle" behind neurogenesis and morphogenesis is "assembled" and "disassembled" at a dazzling pace. In contrast, in the adult, neural stem cells and their niches are retained in restricted regions with their local developmental processes occurring for the life (Alvarez-Buylla and Lim 2004).

It should be anticipated that some part of the human population, such as children, pregnant women and groups of hypersensitive persons could be especially sensitive to the NT MW exposures.

XXII. CONCLUSIONS

Non-thermal effects of microwaves depend on variety of biological and physical parameters that should be taken into account in setting the safety standards. These exposures can cause health risk. The current safety standards are insufficient to protect from non-thermal microwave effects. Emerging evidence suggests that the SAR concept, which has been widely adopted for safety standards, is not useful alone for the evaluation of health risks from NT MW of mobile communication. Other parameters of exposure, such as frequency, modulation, duration, dose should be taken into account. New standards should be developed based on knowledge of mechanisms of non-thermal effects. Importantly, because the signals of mobile communication are completely replaced by other signals faster then once per 10 years, duration comparable with latent period, epidemiologic studies cannot provide basement for cancer risk assessment from upcoming new signals. Precautionary Principle should be implemented while new standards are in progress. In many cases, because of ELF modulation and additional ELF fields created by the MW sources, for example by mobile phones, it is difficult to distinguish the effects of exposures to ELF and MW. Therefore, these combined exposures and their possible cancer risks should be considered in combination. It should be anticipated that some part of the human population, such as children, pregnant women and groups of hypersensitive persons could be especially sensitive to the non-thermal microwave exposures.

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Plausible Genetic and Metabolic Mechanisms for the Bioeffects of Very Weak ELF Magnetic Fields on Living Tissues

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I. INTRODUCTION

A. The “kT Problem”

The biological effects of weak extremely-low frequency (ELF) magnetic fields (MFs) have long been a subject of controversy, with many expressing skepticism as to their very existence: ELF-MFs have lacked a credible mechanism of interaction between MFs and living material.

A prominent conceptual objection has been the “kT problem” (Binhi, 2007). This “problem” can be summarized by the very large ratio between the energy available from a quantum of ELF radiation (2.47×10^{-13} eV) and the thresholds for ionization of atoms (4.34 eV for potassium), chemical activation (~ 0.7 eV), or even the 0.156 eV able to transfer protons across gA channels (Chernyshev, 2002).

What these numbers show is that ELF MFs are certainly not able to have effects through these particular mechanisms, but a detailed theoretical analysis (Binhi, 2007) does not preclude that ELF-MF effects could occur in other ways. MFs can alter the shape of the orbitals of particles without substantially altering their energies, possibly leading to very low thresholds for MF biological effects. Rather than a pure energy problem, as stated above, the true “problem” is to determine if biological structures exist that can be disturbed by very low-amplitude ELF MFs.

II. KEY SCIENTIFIC EVIDENCE

B. Magnetic Sensors

Modern electronics provides interesting examples, such as the MOSFET, where tiny signals can control large energies: a voltage applied to a gate with nominally zero current allows control of substantial drain currents. Biological systems have their own sources of energy, and the MF need only contribute a perturbing influence.

In the context of ELF MF effects, it is useful to examine the transducers of MF-measuring instruments. Induction coils have long been the item of choice for many such instruments, but they suffer from a lack of analogy with possible biological equivalents, in that they gather signal from substantial surfaces (the coil core), and then concentrate the action of the magnetic flux variations gathered over that considerable area at a single point, the contact of the winding.

Hall-effect probes are closer to the mark, in that they detect the potential difference created by a MF on a current flowing in a semi-conductor. Here, the MF acts to deflect a current flow that is powered by an extraneous source. This device dissociates the energy available from the MF itself from the energy it controls.

Another electronic device even closer to the biological transducer we seek is the Spin Tunnel Junction (Micromagnetics, 2012). Such a junction is made of two ferromagnetic metal layers separated by an insulating barrier of a few nanometers (Fig. 6). If a small voltage is applied across the junction, electrons will tunnel through the barrier, according to the ambient MF. The device's MF sensitivity is based on spin-coherent tunneling: the probability of an electron tunneling across the barrier is dependent on its spin, because an electron of a given spin must tunnel to an unfilled state of the same spin. Even the simplest free-electron descriptions of Spin Polarization and Tunneling MagnetoResistance confirm that junction characteristics are determined not only by the ferromagnetic layers, but depend as well on the properties of the barrier (Tsymbal, 2003). Solid-state Spin Tunnel Junctions can detect MFs as low as 0.26 nT at 60-Hz. What these solid-state devices demonstrate is that very small MFs can have effects within the bulk of materials, and that changes in the properties of insulating materials can affect electron tunneling.

C. Magnetic Fields and Incubators

MF experiments with living cells are immediately faced with a practical problem. Cell culture incubators have within them relatively large MFs, due to their relatively weak attenuation of environmental MFs, and to the necessity of implementing controlled heating, humidity and CO_2 concentration conditions. The first control simulates body temperature, the second avoids osmotic imbalance through evaporation, and the third stabilizes pH values within cell culture media. Table 1 was compiled in a survey of 46 incubators used in research (Su, 2012), and showed that average MFs in water-jacketed CO_2 incubators range from 0.9 to 13 µT.

The reaction of many investigators to this situation has been to compensate for the high backgrounds by using even larger MFs in their experiments. According to the conventional dose-responses expected in Toxicology, the effect of an agent can be detected even in the presence of a background exposure, since the biological response is expected to rise smoothly with dose. Many investigators must also have felt that more robust data would be obtained using larger exposures, and that background MFs in incubators could be tolerated.

Table 1. Summary MF Table of 46 Surveyed Incubators (in µT).

Brand	Model	Type	Mean	Min	Max	Max Background
New Brunswick	G-25	Shaker	**0.39**	0.2	0.81	2.06*
Chicago Surgical Ele.	N.A.	General	**0.61**	0.25	1.21	3.32*
Forma Scientific	3956	General	**0.76**	0.2	2.64	0.22
Fisher Sci.	Isotemp	General	**0.76**	0.05	1.85	0.32
Fisher Sci.	637D	General	**0.84**	0.22	2.49	0.23
Forma Scientific	3157	CO_2 W	**0.91**	0.11	2.66	1.77*
Thermo Electron	N.A.	Shaker	**0.98**	0.57	1.58	5.86*
Nuaire	US auto flow	CO_2 W	**0.99**	0.4	2.28	1.34*
Thermo Forma	3310	CO_2 W	**1.04**	0.32	3.75	0.68*
Innova New Brunswick	4200	Shaker	**1.17**	0.31	2.97	0.4
Fisher Isotemp	281	General	**1.86**	1.2	2.22	0.47
Baxter	WJ501	CO_2 W	**1.87**	0.77	5.27	1.6*
Sanyo	N.A.	CO_2	**2.77**	0.85	6.72	0.3
New Brunswick	G-25	Shaker	**2.79**	0.42	16.13	0.31
Sanyo O_2/ CO_2	MCO-18M	CO_2	**2.8**	1.48	4.14	0.81*
Sanyo	MCO_19AIC	CO_2	**2.94**	1.63	5.17	3.31*
Sanyo	MCO-20AIC	CO_2	**3.12**	1.22	6.64	6.68*
Hera Cell	240	CO_2	**3.28**	2.36	4.62	1.48*
Baxter	Tempcon	General	**3.36**	0.61	7.43	1*
Innova New Brunswick	4000	Shaker	**3.47**	1.27	9.53	0.36
Hera Cell	N.A.	CO_2	**3.65**	2.68	4.49	0.26*
Thermo Scientific	370	CO_2	**3.84**	1.9	7.01	0.64*
New Brunswick	C25	Shaker	**3.88**	0.33	17.74	0.96*
Thermo Electron	3110	CO_2 W	**3.91**	1.19	8.56	0.92*
Nuaire	Nu4750	CO_2 W	**3.95**	0.77	10.38	0.64*
Thermo Scientific	370	CO_2	**3.99**	2.03	6.25	0.96*
Forma Scientific	3130	CO_2 W	**4.67**	1.53	11.14	1.37*
Forma Scientific	3110	CO_2 W	**5.44**	1.77	12.59	2.42*
Fisher Sci.	546	CO_2 W	**6.58**	2.36	16.88	0.38
Forma Scientific	N.A.(Old)	CO_2	**6.71**	2.32	16.83	1.36*
Thermo Electron	3130	CO_2 W	**6.79**	1.73	16.97	18.9***
Thermo Electron	3110	CO_2	**7.55**	1.83	18.28	3.92*
Revco	N.A.(Old)	CO_2	**7.67**	3.57	17.76	1.27*
Napco	3550	CO_2	**7.8**	3.52	13.42	2.84*
Thermo Electron	Napco 3550	CO2	**7.83**	3.81	12.13	1.63*
Fisher Sci.	Isotemp 546	CO_2 W	**9.61**	2.34	37.58	0.76*
Thermo Forma	3110	CO_2 W	**9.73**	2.73	24.14	0.47*
N.A.	N.A.	General	**10.46**	3.57	19.51	0.2

Thermo Forma	3110	CO_2 W	**11.89**	3.3	30.41	0.49*
Gallenkamp	N.A.	General	**11.96**	3.06	37.17	2.3*
Fisher Sci.	610	CO_2	**12.3**	5.15	35.52	1.59*
Forma Scientific	3158	CO_2 W	**13.08**	2.62	50.64	1.61*
Labline	3527	Shaker	**14.04**	3.62	42.74	11.87**
WWR international	2005	General	**15.48**	4.92	47.37	1.28
Forma Scientific	546	CO_2	**16.5**	2.61	74.47	3.45*
Sanyo	MIR152	CO_2	**26.98**	5.67	120	0.34*

Type “CO_2 W” means CO_2 incubator with water jacket. “Max Background” refers to measurements outside the incubators. * measured at 50 cm or halfway between the incubator and other electric equipment. ** 5 cm to another incubator. *** 10 cm to a power outlet panel. For more details, refer to Dong and Héroux, 2012.

D. Magnetic Shielding

If it is desired to eliminate the background MFs of incubators to low levels, shielding must be implemented within the incubators. We achieved this in our own experiments using structural steel cylinders 6.3 mm in thickness. As shown in Fig. 1, culture vessels are centered in concentric rectangular structural steel pipes 5.1 x 7.6 x 20 cm, 7.6 x 10.2 x 20 cm and 15.2 x 24.5 x 36 cm. This configuration reduces 60-Hz MFs by a factor of 144, providing “unexposed” cells with a MF environment of 3 nT, slightly below the measurement floor (5 nT at 60-Hz) of our Narda EFA-300 MF instrument (Li, 2012a). The shielding weighs about 20 kg, and is subject to corrosion, if used in the incubator for long periods of time. Fig. 2 shows the change along the axis of the shielding in the triaxially integrated MF. Static MFs within the shields are slightly lower than 50 µT, as structural steel is de-magnetized during production, but of random direction.

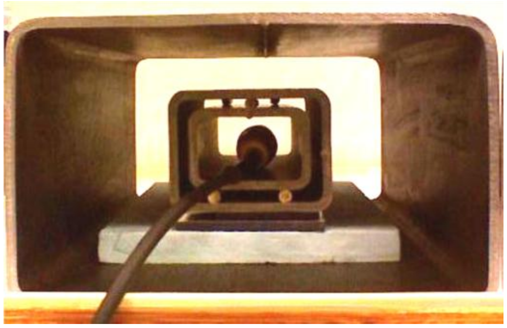

Fig. 1. The three layers of magnetic shielding. The Narda EFA-300’s MF probe is in place of the culture vessel. MF coils for exposure are below, but not in contact with the two smaller shields, insulated from the outer shield by a layer of rigid foam.

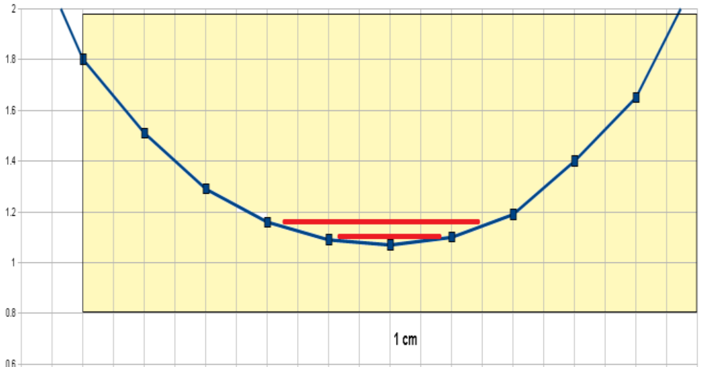

Fig. 2. MF density (μT) generated by an exposure coil vs longitudinal distance inside a magnetic shield pair. The two red lines show the extent of T-25 and T-12 culture vessels, and the yellow rectangle is the smaller shield outline.

E. Experiments on Cells

We conducted experiments on 5 cancer cell lines, with the objective of bringing high precision to our *in vitro* determinations. This objective was reached using automated data acquisition and real-time computer vision, which allowed automated recognition of cells, apobodies and decay particles in cell cultures (Héroux, 2004). In order to reduce deviations related to changing cell culture media, our work used a single synthetic medium (rather than Fetal Bovine Serum) for all 5 cancer models investigated (Li, 2012b).

We first focused our work on changes in the behavior of our cell models under various levels of oxygen. Somewhat surprisingly, all 5 models survived even under anoxic (0 % oxygen) conditions, confirming the exceptional flexibility of cancers cells, able to thrive under anoxia, presumably by finding glycolysis-based sources of cellular energy even in the absence of oxygen.

Low oxygen conditions are actually quite representative of the normal environment of many cells in the body, and are certainly a better *in vitro* representation of the environment of tumor cells, which grow in oxygen and nutrient-restricted environments.

Withdrawal of oxygen suppresses metabolism, as a major combustible of mitochondrial ATP synthesis, oxygen, is eliminated. Metabolism can also be suppressed by a number of chemicals such as oligomycin, imatinib and melatonin-vitamin C, which we collectively designated as “metabolic restrictors”.

F. Karyotype Contraction

When grown under *anoxia* (as opposed to *atmoxia* which is 21 % oxygen, and the commonly used cell culture condition) our 5 cancer cell models lost 6 to 8 chromosomes from their normal number (Table 3). Further, in the presence of strong doses of antioxidant metabolic restrictors, the cell lines quickly reverted to almost normal chromosome numbers (47 – 49). The anoxic cells showed increases in proliferation rate, and the acquisition of a stable, stem phenotype.

Using our 5 hyperploid (54 – 69 chromosomes) cancer cell models, we found that our cells adjusted their chromosome numbers up or down, to match their micro-environment, through rapid mechanisms of endo-reduplication (unscheduled, extra-mitotic chromosome duplication) or chromosome loss. We called this reversible loss of chromosomes under suppressed metabolism "Karyotype Contraction" (KC).

Anoxic K562 displays a very stable karyotype, with 75 % of the cells having either 61 or 62 chromosomes. With the knowledge that metabolic changes would change these chromosome counts, we then set out to investigate the effects of ELF MFs on this model, while we carefully controlled MFs using the shielding techniques described above. We were then using KC as a metabolic scale.

Starting from cell cultures maintained in a pre-industrial environment (less than 4 nT 60-Hz MF), our 5 cancer cell lines were exposed to constant ELF-MFs within the range of 0.025 to 5 µT, and the cells were examined for karyotype changes after 6 days.

As shown in Table 2, all cancer cells lines lost chromosomes from MF exposures, with a mostly flat dose-response. It seemed that the number of chromosomes lost was more specifically connected to the particular cell type than to the MF level, although the two erythro-leukemia cell types both showed a dose-response between 25 and 400 nT.

Surprisingly, constant MF exposures for three weeks allowed a rising return to the baseline, unperturbed karyotypes. From this point, small MF increases or decreases (10 %) were then again capable of inducing karyotype contractions (Li, 2012a).

Table 2. Karyotype Contraction (mean number of chromosomes lost over 6 days)

Magnetic Field (nT)	Anoxic K562 Erythroleukemia	Atmoxic HEL Erythroleukemia	Atmoxic NCI-H460 Lung cancer	Anoxic MCF-7 Breast cancer	Atmoxic COLO-320DM Colon cancer
25	2.21				
50	4.92	10.22	7.52	11	5.36
100	8.18	11.55			
200	11.04				

400	10.4	12.79	7.55	10.64	5.85
700	9.52				
1000	7.69			10.68	
1500	9.94				
5000	12.1	13.03	7.46	10.95	5.78

Table 3. Karyotype Contraction (mean number of chromosomes lost over 6 days)

Cell	**Atmoxic Modal Chromosome Number**	**Anoxic KC**	**Anoxic to MF Saturation KC**	**Atmoxic to MF Saturation KC**	**Atmoxic to Anti-Oxidant Suppression KC***
K-562 Erythroleukemia	69	7	10.12		21.34
HEL Erythroleukemia	66	7		12.91	18
MCF-7 Breast cancer	82	8	10.82		18
NCI-H460 Lung cancer	57	6		7.51	10
COLO-320DM Colon cancer	54	6		5.66	7.7
Average	*65.6*	*6.8*	*10.47*	*8.69*	*15.01*
Condition	+ O_2	- O_2	- O_2 + MFs	O_2 + MFs	O_2 + Oxidative Inhibition

The conclusion from these observations was that MFs act as a metabolic inhibitor, even at very low levels commonly encountered in the normal environment.

G. ATP Synthase

Supplementary tests carried out by comparing MF-exposed cell cultures to cultures exposed to various metabolic suppressors showed that the MF-exposed cultures were remarkably similar to those exposed to oligomycin A, a specific inhibitor of the Fo segment of the enzyme ATP Synthase (ATPS).

But how could MFs as low as 25 nT alter the activity of ATPS? ATPS has the structure of a motor-generator than normally produces ATP using the energy of a flow of protons through a turbine-like structure, Fo. MFs apparently impaired the flow of protons through ATPS Fo.

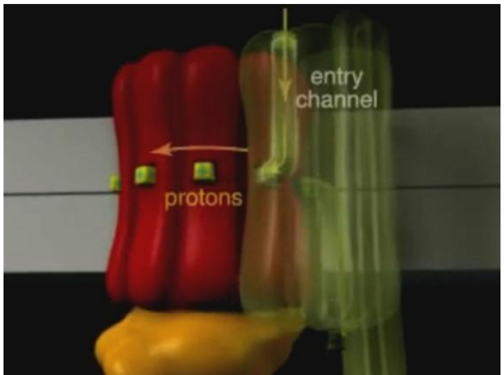

Fig. 3. The structure of ATPS Fo: entry and exit channels for the movement of protons (Yoshida, Tokyo Institute of Technology).

Russian physicists (Semikhina 1981; Semikhina 1988) have reported that very low levels of ELF MFs (25 nT) can alter the structure of water, and that the effects of the altered water structure would be particularly important under high concentrations of protons and water molecules. An interesting aspect of these changes in water structure is that the transition between states takes several hours.

As it turns out, the entry and exit channels of ATPS Fo (Fig. 3) are hydrophilic channels, which means that they are expected to be filled with water molecules, and the intermembrane potential of mitochondria maintains a large electric field (180 kV/cm) which concentrates protons within them. These locations seem ideal to embody the low level effects documented by Semikhina and Kiselev.

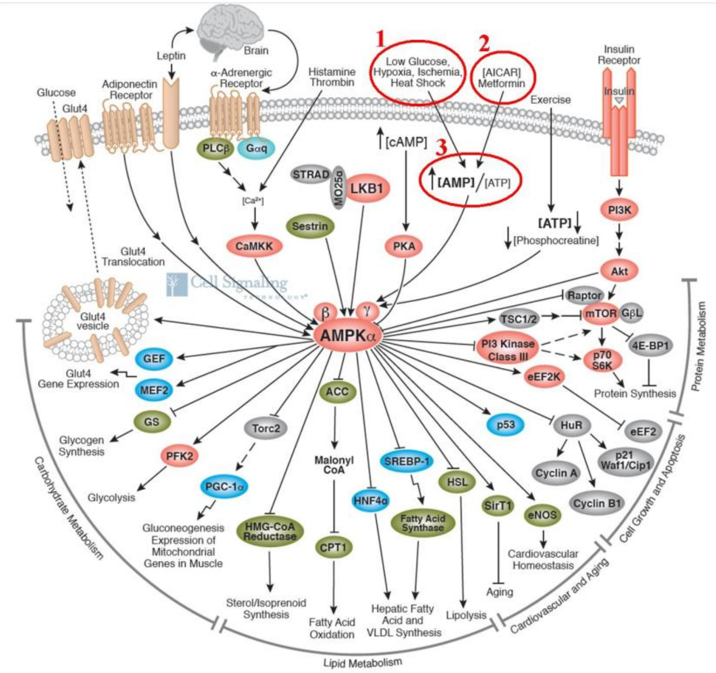
Brain
Leptin
Glucose
Glut4
Adiponectin Receptor
α-Adrenergic Receptor
Histamine Thrombin
1
Low Glucose, Hypoxia, Ischemia, Heat Shock
2
[AICAR] Metformin
Exercise
Insulin Receptor
Insulin
PLCβ
Gαq
[Ca2+]
STRAD
MO25α
LKB1
↑[cAMP]
3
↑[AMP]/[ATP]
PI3K
[ATP]↓
↓[Phosphocreatine]
CaMKK
Sestrin
PKA
Akt
Glut4 Translocation
Cell Signaling Technology
Raptor
mTOR
GβL
TSC1/2
β
γ
Glut4 vesicle
AMPKα
PI3 Kinase Class III
p70 S6K
4E-BP1
eEF2K
Protein Synthesis
Protein Metabolism
GEF
Glut4 Gene Expression
MEF2
GS
ACC
Torc2
p53
HuR
eEF2
Glycogen Synthesis
PFK2
Malonyl CoA
SREBP-1
p21 Waf1/Cip1
HSL
Cyclin A
PGC-1α
HNF4α
Glycolysis
SirT1
Cyclin B1
eNOS
Fatty Acid Synthase
Gluconeogenesis Expression of Mitochondrial Genes in Muscle
HMG-CoA Reductase
CPT1
Cardiovascular Homeostasis
Carbohydrate Metabolism
Aging
Cell Growth and Apoptosis
Sterol/Isoprenoid Synthesis
Hepatic Fatty Acid and VLDL Synthesis
Lipolysis
Fatty Acid Oxidation
Cardiovascular and Aging
Lipid Metabolism

Fig. 4. The many regulatory pathways of AMPK, with the hypoxic (1), metformin (2) and ATPS suppression sites (3) labeled (http://www.cellsignal.com/).

H. AMPK

If the mechanism was indeed as we thought, then MFs would alter the production of ATP in cells. If this happened, another important intracellular enzyme, AMP-activated protein kinase (AMPK), would immediately be activated, as AMPK is extremely sensitive to changes in the level of ATP. We tested this hypothesis by two supplementary assays involving metformin and resistin. As expected, MF effects were amplified by metformin, an AMPK stimulator, and attenuated by resistin, an AMPK inhibitor (Li, 2012a).

Our data therefore suggests that the karyotype contractions caused by MFs stem from interference with mitochondria’s ATP synthase (ATPS), compensated by the action of AMPK. The involvement of AMPK also conveniently explains the slow restoration of karyotypes to their original level after 3 weeks, as AMPK is not only fast-acting to restore ATP levels, but slow-acting through its numerous metabolic and genetic regulation pathways (Fig. 4). It may also explain the unusual observation where increases or decreases in MF exposures can both produce KCs (Li, 2012a).

I. In the Channels

Some enzymes operate faster than predicted by classical thermodynamics, and their increased speed can be explained by tunneling of protons or electrons through activation barriers (Garcia-Viloca, 2004; Olsson, 2004). Quantum tunneling for protons over 6 nm through bridging by water molecules has been observed in tryptamine oxidation by aromatic amine dehydrogenase, for example, and tunneling in enzymatic reactions is now widely accepted in biological models (Masgrau, 2006).

It is of interest to examine how protons may flow through ATPS Fo channels. The protons trickle through a thin pipe of water molecules, propelled by an electric field of about 180 kV/cm. Adiabatic tunneling should be more efficient than non-adiabatic coupling, implying that disturbances along the channel could result in loss of channel transparency. Proton-coupled electron transfer underpins many biological reactions, and may occur as unidirectional or bidirectional, and synchronous or asynchronous, transfer of protons and electrons (Reece, 2009).

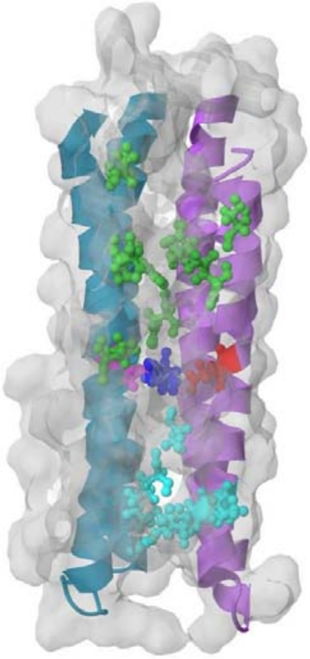

Fig. 5. The ATPS Fo proton hydrophilic channel. Hydrophilic side chains and residues are in green and blue. (from Sasada R, Marcey D. ATP Synthase, 2010. http://www.callutheran.edu/BioDev/omm/jmolxx/atp_synthase/atp_synthase.html#fig1).

It is probable that both electrons and protons tunnel through the channel, making theoretical analysis more complex, especially as electrons meet with different protons along a chain. Since protons are much heavier than electrons (x1836), their wavelength is 43 times shorter (inverse square root), and electrons may transfer over longer distances (Moser, 1992; Gray, 1996). Thus, electron transfer can span fractions of nano-meters, while proton transfer occurs mostly within a hydrogen bond (less than 0.197 nm). The hydrogen bond strength (23.3 kJ/mol) is just 5 times the average thermal fluctuation energy. Quantum chemical calculations show that this strength can vary as much as 90 %, depending on the level of cooperativity or anti-cooperativity within water molecule chains, which corresponds to a bond length change of 9 %, or 0.018 nm (Hus, 2012).

This limited reach of proton tunneling and its delicate dependence on water cluster structure may be major factors underlying the sensitivity of ATPS performance to MF-exposed water.

J. Water 'Remanence'

From our observations, particularly the fact that exposed cell culture medium can retain memory of past MF exposures (Li, 2012a), it does not appear that biological effects of MFs, as we detected them, are based on a direct interaction with electrons or protons, but rather, as suggested by Semikhina and Kiselev, on an interaction between MF and the structure of water, which in turn influences electron and proton tunneling. The exact structure of the water molecule arrays responsible is not known, but may be connected with long-lived hydrogen bond structures which confer particular proton transparency to ATPS Fo water channels. This structure seems vulnerable to interference by MFs over a wide range of intensities and possibly frequencies (Kiselev, 1988). Perturbations to the structure of O-H bond vibrations has even been spectroscopically detected as slow (hours) transitions in water exposed to sunlight radiation (Yokono, 2009).

This would not be the first instance of subtle changes in hydrogen bonds resulting in large influences in biology. A contemporary example relates to the selective uptake of phosphorus rather than arsenic by bacteria. The discrimination by a factor of 4,500 in phosphorus vs arsenic is based on a 4 %

distortion in a unique low-barrier hydrogen bond (Elias, 2012).

III. DISCUSSION

There are similarities as well as differences between semi-conductor tunneling and ATPS tunneling. Both involve oxygen; tunneling distances, as well as the voltages applied (Fig. 6) are similar. But in semiconductor tunneling, only electrons are mobile, while protons move within ATPS. In the semiconductor, magnetic sensing is mainly through shifts in the populations of electrons with a given spin, determined by the electrodes. In ATPS, the transparency of the water channel seems determined by long-term MF exposures.

Perhaps least understood is how cells can metabolically compensate for various MF exposures over time, as shown by the restoration of their chromosome numbers after three week exposures (Li, 2012a). Anoxia leads to permanent KCs, but other KCs from MFs or other anti-oxidants are transient. Most anti-oxidant and MF KCs are larger than the atmoxic to anoxic transition KCs, possibly because some oxygen is still available to cell metabolism, even under anoxic conditions. Anoxia and MFs together are effective metabolic suppressors.

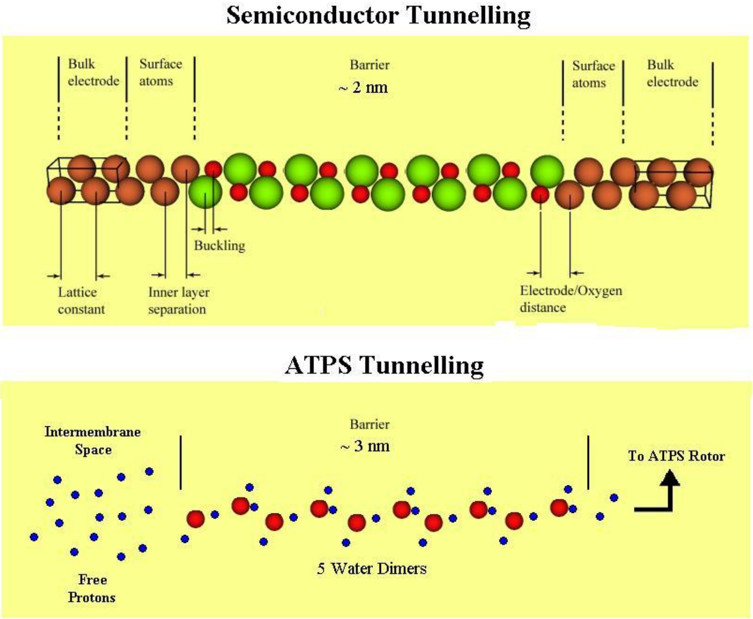
Semiconductor Tunnelling
Bulk electrode
Surface atoms
Barrier ~ 2 nm
Surface atoms
Bulk electrode
Buckling
Lattice constant
Inner layer separation
Electrode/Oxygen distance
ATPS Tunnelling
Intermembrane Space
Barrier ~ 3 nm
To ATPS Rotor
5 Water Dimers
Free Protons

Fig. 6 Tunneling in magnetic sensors and in ATPS water channels.

IV. CONCLUSIONS

The particularities of hydrogen bond structures in water can justify the subtle changes detected in water structure under MF exposures. Under specific circumstances, such water changes may influence the flux of protons in ATPS channels, thus inducing some biological effects of MFs. These interactions seem to involve very small energies, and also seem to require hours to establish themselves, thus bypassing the celebrated "kT problem". These results may be environmentally important, in view of the central roles played in human physiology by ATPS and AMPK, particularly in their links to diabetes, cancer and longevity (Li, 2012a). The wide range of MF amplitudes and frequencies that can potentially disturb ATPS make this effect a global health issue. Although society seems to compile diseases with more enthusiasm that longevity (Li, 2012a), it should be remembered that MF exposures may have both undesirable and desirable effects on health.

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SECTION 17

Evidence based on EMF Medical Therapeutics

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Prepared for the BioInitiative Working Group
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I. Introduction

Electromagnetic fields are widely used in therapeutic medical applications. Proof of effectiveness has been demonstrated in numerous clinical applications of low-intensity ELF-EMF and RF-EMF, each treatment employing specific characteristics of frequency, modulation and intensity to achieve its efficacy. On the other hand, higher levels of EMFs encountered in the environment which are indiscriminately generated by technologies of the 20th and 21st centuries may result in harm. EMF levels which are allowable today under thermally-based public exposure standards do not take into account these clear indications of the sensitivities of the human body to EMFs. If we are to promulgate public exposure standards that are protective of public health, then this body of evidence on healing with EMFs is of primary importance in developing biologically-based public exposure standards.

> *"Although incompletely understood, tissue free radical interactions may extend to zero field levels. Emergent concepts of tissue thresholds to imposed and intrinsic magnetic fields address ensemble or domain functions of populations of cells, cooperatively whispering together in intercellular communication and organized hierarchically at atomic and molecular levels." 10*

II. Therapeutic Uses for Electromagnetic Fields

Since EMFs have been shown to be effective in treating conditions of disease at energy levels far below current public exposure standards, this body of evidence forms a strong warning that indiscriminate EMF exposure is ill advised. Health concerns from indiscriminate exposure to EMF, as opposed to EMF exposures done with clinical oversight, could lead to harm as can the unsupervised use of pharmaceutical drugs.

The consequence of multiple sources of EMF exposure in daily life, with no regard to cumulative exposures or to potentially harmful combinations of EMF exposures will pose future difficulties in identifying sources of disease (because of multiple and overlapping exposures) and time-varying and geography-varying differences from person to person.

Just as ionizing radiation can be used to effectively diagnose disease and treat cancer, it is also a cause of cancer under different exposure conditions. Since EMFs are both a cause of disease, and also used for treatment of disease, it is vitally important that public exposure standards reflect our current understanding of the biological potency of EMF exposures.

"there is an abundance of experimental and clinical data demonstrating that exogenous EMFs of surprisingly low levels can have a profound effect on a large variety of biological systems. Both electrical and electromagnetic devices have been demonstrated to positively affect the healing process in fresh fractures, delayed and nonunions, osteotomies, and spine fusion in orthopedics and for chronic and acute wound repair. These clinical results have been validated by well-designed and statistically powered double-blind clinical trials and have survived meta-analyses. The FDA has approved labeling for these biophysical devices, limited at present to these indications." "The potential clinical applications of EMF therapeutics extend far beyond those considered here and the clinical rewards are certain to be huge." "Cancer, cardiac muscle regeneration, diabetes, arthritis, and neurological disorders are just some of the pathologies that have already been shown to be responsive to EMF therapy. Successful applications of low-frequency EMFs have been reported for treatment of bronchial asthma, myocardial infarction, and venous and varicose ulcers. There is emerging research on EMF effects on angiogenesis and the manner in which this may increase stem cell survival in the treatment of Altzeimer's (sic) and Parkinson's diseases. There are also many studies that point to the possibility of the use of EMF for peripheral nerve regeneration" and *" the treatment of cancer." "EMF therapy modalities are simple, safe and significantly less costly to the health care system. They offer the ability to treat the underlying pathology rather than simply the symptoms. The time is particularly opportune given the increased incidence of side effects from the use of pharmacological agents. EMF therapeutics will have a profound impact upon health and wellness and their costs worldwide."* [1]

A. Bone Repair

Clinical use of pulsed EMF has been demonstrated to achieve bone repair, particularly in fractures that do not heal on their own. Bone healing is stimulated by very weak electromagnetic fields that are far lower in strength than would produce tissue heating. The FDA approved pulsed EMF for use in bone healing in 1979. Since that time, many millions of patients have benefited from this therapy. Since PEMF treatments are non-invasive and clinically effective, it has advantages to the patient in terms of reduced pain and suffering, reduction in health care costs, and effectiveness where other methods have failed to produce adequate clinical results.

"It is now commonplace to learn the successful use of weak, nonthermal electromagnetic fields (EMF) in the quest to heal, or relieve the symptoms of a variety of debilitating ailments. This chapter attempts to give the reader an introduction and assessment of EMF modalities that have demonstrated therapeutic benefit for bone and wound repair and chronic and acute pain." [2]

Pilla provides extensive discussion of the "clinical evidence that time-varying magnetic fields (EMF) can modulate molecular, cellular and tissue functions in a physiologically significant manner." [2] A description of the various waveforms and EMF modalities which are effective in bone and wound repair are beyond the scope of this paper, but are well documented. [2] In addition to documenting that bone repair in fractures is achieved with pulsed EMF at low intensities, Pilla also reports that pulsed EMF has been successful in promoting bone repair and healing of spine fusions for the treatment of chronic back pain from worn and/or damaged spinal discs. [3] The FDA has approved pulsed EMFs for bone healing and this is a widely recognized treatment, particularly for fractures that are slow to heal, or do not repair with conventional medical treatment. It represents one of the best documented cases in science where the body clearly responds to low-intensity EMF signals for healing purposes; these EMF signals are far below current public exposure standards and are proof of the bioactivity (in a beneficial form as applied).

Liboff describes signal shapes in electromagnetic therapies that contribute greatly to our understanding of the various forms of EMF signal delivery that are fundamental to eliciting specific bioeffects. He simply and elegantly describes electric and magnetic signal characteristics, their signature shapes and methods of delivery (time-varying, oscillatory, or modulated) which create special interactions with human tissues and organs for healing. [4]

"It is likely that the future will see combinations of such signals in therapeutic applications, especially as more information filters back from the laboratory elaborating on the nature of electromagnetic interactions with living tissue." [4]

B. Wound Repair

The clinical application of pulsed EMF has been shown to enhance wound repair and healing. [2,5] Devices that use pulsed EMF have been approved for use in the United States by the FDA. Pilla reports "*the clear clinical effectiveness of PEMF signals has resulted in significantly increased use"* in treating wounds that do not heal. [5] In Pilla's extensive summary presented on beneficial effects of EMF on wound healing, he reports pulsed EMF has been reported to reduce edema, increase blood flow, modulate upregulated growth factor receptors, enhance neutrophil and macrophage attraction and epidermal cell migration, and increase fibroblast and granulation tissue proliferation. Most wound studies were conducted on arterial or venous skin ulcers, diabetic ulcers, pressure ulcers, and surgical and burn wounds. [5] Wound repair under the influence of very low level pulsed EMFs is a second solid documentation in science that very low level EMFs are bioactive (in this case, beneficial) when applied in very specific clinical applications where the exposure variables are carefully selected.

Oschman provides an overview of the evolution of energy medicine and electromagnetic energy treatments related to bone repair, wound healing, pain relief, depression, insomnia, inflammation of tissues and other medical conditions. [6] He also underscores the counter-intuitive thesis that low-intensity EMFs can be more effective in eliciting healing responses than larger intensity exposures; and that understanding of the subtle energies and their specific interactions with human functioning is imperative.

> *(l)iving tissues are far more sensitive to external fields than previously realized. After a period when physicists were certain that observed sensitivities to nonionizing and nonthermal radiations wer physically impossible, we now know that biological systems defy the simple logic that larger stimuli should produce larger responses.For many living systems, extremely weak fields can be more effective than strong fields."* [6]

C. Pain Management

Pulsed magnetic field (PMF) devices are also used with FDA approval for *"relief of acute and chronic pain and the reduction of edema* (swelling*), all symptoms of wounds from post-surgical procedures, musculoskeletal injuries, muscle and joint overuse, as well as for chronic wounds."* [5]

Pulsed EMF has been shown to be effective in relief of chronic pain associated with connective tissue injury (cartilage, tendon, ligaments and bone) and soft-tissue injuries associated with the joints. Both acute and chronic pain may be successfully treated with EMFs as an alternative to non-steroidal anti-inflammatory drugs (NSAIDs). Relief from chronic pain due to osteoarthritis has been reported with treatment by EMFs. [2]

Markov reports that EMF is used in treatment of pain associated with tendonitis, multiple sclerosis, carpal tunnel syndrome and some forms of arthritis. He discusses the use of pulsed EMF for headache and migraine pain relief; neck and whiplash injuries, postoperative pain, sprains, chronic pelvic pain, and nerve regeneration. Pain reduction by clinical application of pulsed EMF is achieved with non-thermal levels of exposure, and produces a nonthermal biological effect. [8]

D. Depression, Anxiety Disorders, Insomnia

"Today (2002) we are at a threshold for the acceptance of electromagnetic therapy as a clinically accepted form of therapy for such diverse diseases as unipolar depression, Parkinson's disease, and sleep disorders and the treatment of debilitating chronic and acute pain." [8]

Shealy et al (2007) detail clinical findings for treatment of depression and mood management, reduction in anxiety, and treatment of insomnia. 10 Electrical energy stimulators that deliver very low-level EMF have been reported to be clinically effective in the alteration of neurobioechemicals including serotonin and cortisol. Depression, mood disorders and insomnia have been related to disregulation of serotonin levels.
Use of EMFs to reduce symptoms of depression, anxiety and insomnia are authorized by the FDA, and have been in use since the 1970's. Shealy reports that transcranial stimulation by EMFs led to a significant relief of depression in 85% of patients who had failed pharmacological

agents, and was at least twice as effective as any known antidepressant drugs and without complications. [10]

E. Protection from Anoxia (Protection for the Heart)

The work of Albertini, Litovitz and di Carlo, Goodman and Blank, Han, Pipkin, Rasmark and Kwee, [11-17] has shown that very weak ELF-EMF and RF-EMF exposures can actually help to protect cells against tissue damage. They can induce an adaptive stress response in cells, which in turn helps the cell fight damage. The response is production of stress proteins (heat shock proteins or HSP). These stress proteins help to protect the cells against injury and death. A 20-minute exposure to electromagnetic fields at only 80 mG will start stress protein production, which helps to fight cellular damage from lack of oxygen, for example. Protection from anoxia (or lack of oxygen) is important in heart attacks. Pre-treatment with ELF-EMF (and also RF-ELF) before blocking oxygen to cells has been shown to be protective against the lack of oxygen to heart tissues. The exposure level is on the order of 80 mG ELF-EMF or far below any possible thermal heating.

This means that there are clinical applications for protection against heart attack damage that can be provided by very low-dose EMF exposures. Such protection could be vitally important in reducing damage from oxygen loss during heart attacks. It is another line of proof that low-intensity electromagnetic fields are bioactive, and when applied in specific therapeutic ways, are beneficial. It also underscores that the body can detect and decode these very weak signals, providing further evidence that thermally-based standards are incomplete because they do not take into account the sensitivity of the human body to non-thermal levels of EMF exposure.

IV. Conclusions

Since EMFs have been shown to be effective in treating conditions of disease at energy levels far below current public exposure standards, this body of evidence forms a strong warning that indiscriminate EMF exposure is ill advised.

Based on extensive clinical applications of low-intensity EMFs since at least the 1970s, it has been demonstrated beyond argument that some forms of EMFs can be medically effective in treating a wide variety of human health disorders and injuries. Since all of these treatments are conducted at energy levels that do not involve tissue heating per se, it is convincing proof that the human body both reacts to and can be affected by exposures to EMFs. Exposures can be beneficial when EMFs are applied with conscious knowledge of the exposure factors that are proven to lead to specific biological (healing) consequences. The intensity of such therapeutic exposures nearly always falls below current public exposure standards as discussed in Section 3.

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SECTION 17

Electromagnetic Medicine
Non-Inductive Non-Thermal Modalities

(Supplement 2012)

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I. INTRODUCTION

The area of electromagnetic medicine (EM) encompasses the applications of electricity and magnetism to medical practice. Although this includes both diagnostic and therapeutic applications, the medical community is far more familiar with the former, notably with techniques such as magnetic resonance imaging (MRI), electromyography (EMG), electroencephalography (EEG), electrocardiography (EKG), and magnetocardiography (MKG). There are historical reasons for the medical unfamiliarity (even antipathy) with electromagnetically-based therapies. One has only to look at the beginnings of modern medicine in the United States, specifically the 1910 Flexner report [1,2] that provided the basis for medical education today. Prior to this report there was widespread use of electromagnetic techniques in medicine, often little more than late 19th century versions of snake-oil cures. In great measure the present aversion to electromagnetic therapies built into modern medicine is a direct result of Victorian age quackery.

Another reason for this antipathy, apart from the constraint on the teaching curriculum, has been the extraordinary success of, first, the germ theories of Pasteur and Koch, and, second, the development of molecular biology following the work of Watson and Crick. These have engendered a sense of completeness, a feeling that there is no place for alternate, radically new approaches to the way that illness is treated. Even when electromagnetically-based therapies have proven beneficial, they have been usually ignored. There is little impetus to replace the existing approach, since it is firmly believed that nothing is more fundamental than the existing paradigm, that questions of wellness and illness are ultimately biochemical in nature.

The divisions in electromagnetic medicine are outlined in Fig. 1. Beyond the separation into diagnostic and therapeutic applications another distinction is made for applications of weak-field ELF magnetic in the treatment of illness. The description ***non-inductive non-thermal*** helps emphasize that the effects obtained by applying low intensity low-frequency electromagnetic fields to biological systems are not the result of either inductive emf generation or the delivery of thermal energies through Joule heating. By contrast, a number of clinical devices that make use of Faraday induction or Joule heating are recognized by the medical community not only because they are effective, but also because the applied voltages, currents or heat are fully consistent with what is expected biochemically. In sharp contrast, the non-inductive non-thermal category includes clinical applications where this is not true, that is, where the electromagnetic variables that are part of the therapy fall outside those permitted by the current medical paradigm.

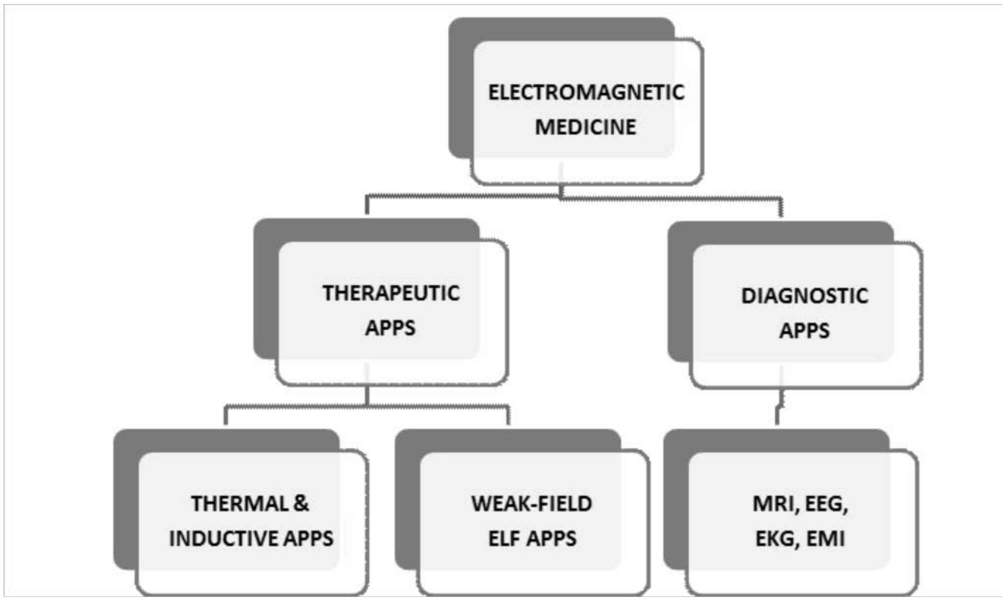

Fig. 1. Divisions comprising Electromagnetic Medicine

II. WEAK-FIELD ELF APPLICATIONS: SCIENTIFIC BASIS

There is a wealth of evidence showing that weakly intense ELF fields affect the metabolic responses in cells. It was found in the 1980s that ELF magnetic fields too weak to be considered as inductive sources of potential differences are nevertheless capable of affecting DNA synthesis in mammalian cell culture[3,4]. Since that time, there have been numerous reports (Table 1) that magnetic fields on the order of several microTesla and in the 3-300 Hz ELF frequency range can affect a wide range of biological systems. A short list of such reports, given in Table 1, emphasizes both the variety of systems in which these effects have been found, and the difficulty in providing an explanation, as evidenced by the fact that these studies have a history extending back more than 25 years. The lack of a reasonable explanation is not a trivial distinction, since there is great reluctance to accept observational evidence, regardless of replications and the number of supportive reports, without a reasonable biomolecular basis

Biological Model	YEAR	Reference
Rat behavior	**1986**	**Thomas et al**[5]
Diatom motility	**1987**	**Smith et al**[6]
Protein synthesis in salivary gland cells	**1988**	**Goodman and Henderson**[7]
Mitogenesis in lymphocytes	**1989**	**Cossarizza et al**[8]
Production of glycosaminoglycans in cartilage	**1991**	**Smith et al**[9]
Neuroblastoma cell metabolism	**1992**	**Smith et al**[10]
Expression of Insulin Growth Factor II	**1995**	**Fitzsimmons et al**[11]
Regeneration of planarians	**1995**	**Jenrow et al**[12]
Analgesia in snails	**1996**	**Prato et al**[13]
Rat EEG	**1998**	**Vorobyov et al**[14]
Growth Rate in plants	**2005**	**Galland and Pazur**[15]
Stem cell differentiation	**2009**	**Gaetini et al**[16]

Table 1. List of reports indicating that non-inductive ELF magnetic fields are biologically interactive. Note that these reports are by no means isolated. A number of these have been independently replicated, for example the studies on rat behavior, lymphocytes, planarians, and plants.

In 1998 a group led by Zhadin[17] discovered that these effects are also found at much lower intensities. AC magnetic fields as low as 40 nT can shift the electrical conductivity of polar amino acids in aqueous solutions. This work, independently replicated[18,19,20], is typified by a sharp change in conductivity at one specific frequency, as shown in Fig. 2. The explanation for this remarkable effect makes use of quantum electrodynamics to provide a means of reducing the viscosity of water sufficiently to allow Lorentz forces to be observed on solvated biological ions, thereby establishing a straightforward reason for the many difficult-to-explain magnetic stimulation reports claiming a connection to ion cyclotron resonance[21].

Ion cyclotron resonance (ICR) as it applies to biological systems was first discovered[22,23] to be a critical underlying factor in connection with previously observed[24] electromagnetically-induced changes in free calcium in brain tissue (Ca-efflux experiments). In the presence of a static magnetic field the most prominent effects are always observed for parallel AC magnetic fields with frequencies very close to the cyclotron frequency of the calcium ion. The majority of subsequent ICR cellular studies have focused on the Ca^{2+} ion. As a second messenger it is involved in regulation at all stages of growth and development, including proliferation, and in the organization of cytoskeletal elements. Indeed some of the results shown in Table 1 are examples of Ca^{2+} ICR stimulation.

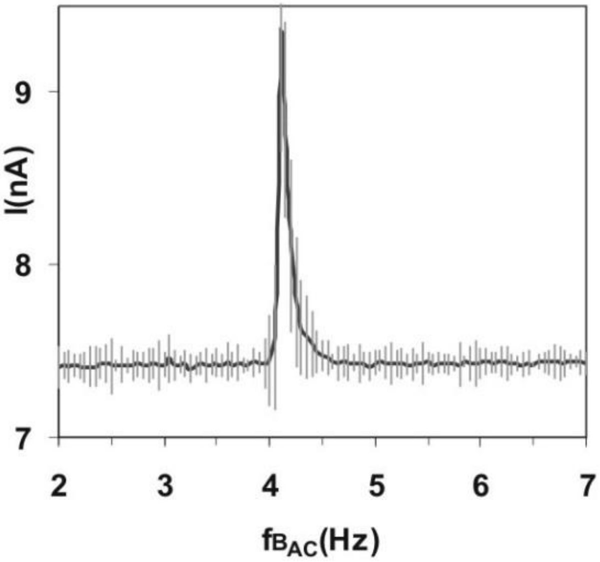

Fig. 2. Data taken by Pazur[18] illustrating the Zhadin effect[17]. A very weak AC magnetic field (40 nT) is applied to an aqueous solution of glutamic acid and the conductivity of the glu$^+$ ions is continuously monitored in terms of nA. The magnetic frequency in Hz is slowly ramped upwards. A sharp change in conductivity is observed at a frequency (4.25 Hz) close to the ion cyclotron resonance value for glu$^+$, (4.8 Hz).

The expression for the ICR resonant angular frequency is given as $w = (q/m)B_o$, where q and m are the charge and mass of the ion, and B_o the DC magnetic field. Confirmation that the charge-to-mass ratio was explicitly involved in this effect was obtained when isotopic $_{45}$Ca was substituted for $_{40}$Ca in a study on lymphocyte proliferation[25], showing that the frequency where the maximum ICR effect on proliferation occurred was shifted down by a factor of 12%, exactly what is to be expected for a change of mass of 5 parts out of 40.

Because these ICR effects appeared to violate simplistic analysis involving magnetic induction at first they evoked much suspicion in the scientific community. Many subsequent confirmations, however, performed on different model systems in diverse experimental situations , in part listed in Table 1, proved that these weak low-frequency effects are indeed real. It is clear that magnetic field combinations when tuned to ion cyclotron resonance, can act to regulate the flow of biological information, a conclusion that has important ramifications for electromagnetic medicine. Consider the following, from a recent review[26] of this subject:

> *The inescapable conclusion…is that the ICR mechanism, whatever its molecular basis, is of enormous biological significance. We are able to make reproducible and consistent physiological changes of various sorts in the widest imaginable range of genera simply by applying weak magnetic fields tuned to the charge-to-mass ratio of various biological ions. It is very clear that [this] must be part of a heretofore unknown system that carries physiological information/instructions, and that better understanding will open the way to providing a radically new means of controlling wellness.*

In addition to medical applications already initiated using ICR techniques there are also a number of potential advances that are likely to be further developed in the future. Consider for example the observations found in a number of ICR studies that indicate merely changing the resonance condition from one ion to another will result in the opposite result. This phenomenon was first observed by S D Smith in his studies on diatom motility[6] and later reported by others[9,27-31] (Table 2). One explanation is that this effect likely reflects the endogenous nature of bioresonance, wherein multiple ion resonances are occurring simultaneously giving rise to a balanced physiologic outcome. If this is true then it should be possible in principle to selectively reduce the undesirable in favor of the desirable. There is evidence[32] indicating that ICR applications can increase the rates of proliferation in neuroblastoma cell culture. Is It possible that there exist yet-to-be-tried ICR conditions that would have the opposite effect, namely to reduce the rates of proliferation in cancer cell lines, thereby opening the way to new cancer fighting techniques?

MODEL SYSTEM	FREQ, Hz	B_o, mT	ION	RESPONSE
Diatom motility[6]	**16**	**20.9**	Ca^{2+}	**Motility✸**
	16	**41.0**	K^{+}	**Motility❋**
Embryonic bone[9]	**16**	**20.9**	Ca^{2+}	**Growth✸**
	16	**40.7**	K^{+}	**Growth❋**
Embryonic bone[27]	**16**	**20.9**	Ca^{2+}	**Growth✸**
	16	**40.7**	K^{+}	**Growth❋**
Plant growth[28,29]	**60**	**78.3**	Ca^{2+}	**Growth✸**
	60	**153.3**	K^{+}	**Growth❋**
Rat behavior [30]	**63**	**50**	Mg^{2+}	**More Active**
	38	**50**	Ca^{2+}	**More Passive**
Gravitropic response[31]	**35.8**	**46.5**	Ca^{2+}	**Up**
	54.7	**46.5**	K^{+}	**Down**

Table 2. Ionic tuning can drastically alter physiological outcome. Note that specific outcomes are observed for different magnetostatic fields at the same resonant frequency, or equivalently, for different frequencies at the same static magnetic intensity.

II. PRESENT CLINICAL ELECTROMAGNETIC PRACTICE

A number of diagnostic techniques based on electromagnetic principles, such as **Magnetic Resonance Imaging** (MRI), are universally accepted by physicians, to the point where objections are heard concerning the costs to the health care system because of overuse[33]. Neurologists universally use **Electromyography** (EMG) in their practice no less than **Electrocardiography** (EKG) is used by cardiologists and internists. It also should be understood that there are efficacious electromagnetic diagnostic tools that are used outside of the United States but not permitted in the US. The US Food and Drug Administration (FDA) oversee the introduction and use of medical devices with as much zeal as it supervises pharmaceuticals. The prospect of very expensive and time-consuming procedures for new devices tends to discourage the introduction of foreign devices, regardless of their efficacy and safety. This applies to both diagnostic and therapeutic devices.

One example of a foreign diagnostic device that is presently in clinical trials in the US is the Tissue Resonance Interferometer (**TrimProbe)**[34], invented by Clarbruno Vedruccio. Following its original use as an electromagnetic device for the remote detection of land mines and for airport screening, he discovered that microwave signals in the range 400 to 1350 MHz reflect differently from cancers as compared with healthy tissue. A hand-held non-invasive probe measures the degree of interference between the incoming and reflected signals, providing instant determinative results. It has been highly successful in prostate diagnosis, proving effective in distinguishing malignancies from prostate hyperplasia and prostatitis. This technique has also been used to detect bladder cancer. Because of its non-invasiveness, its speedy application and rapid diagnosis, all within a matter of minutes, this device has great potential as a tool for screening populations at risk.

It is clearly the case that the highly specific electrical nature of the nervous system should predispose it to exogenous electrical influence. This is shown in the great variety of electric medical procedures[35] presently in use as neurotherapies. Devices such as heart pacemakers and defibrillators are so widely known that they need no description. **Vagal nerve stimulation** (VNS) is widely used as an anti-convulsant therapy. **Deep brain stimulation** (DBS) uses electrodes in the brain to treat Parkinson's disease and other movement disorders. Chronic pain is treated using the non-invasive **Transcutaneous electrical nerve stimulator** (TENS) directly on the back or the **Cranial electrothermal stimulator** (CES) on the head. Insomnia is treated with **Low-energy emission therapy** (LEET) using an electrode positioned in the mouth. In general these devices are employed as surrogates for already existing physiological endogenous mechanisms that require a boost or improvement, with the cardiac pacemaker serving to regulate the timing of heart contractions as an illustrative example. Presently there is an extension of this concept, with widespread ongoing research aimed at mimicking the electric signals needed to restore eyesight and muscle function that may have been lost because of disease or accident.

Less well known are a number of medical accepted EM therapies that are sufficiently energetic to be acknowledged as based either on Faraday induction or Joule heating. **Transcranial Magnetic Stimulation** (rTMS)[36,37] is used to treat depression. In this procedure, approved by the FDA as efficacious and safe, a large pulsed current is sent through a coil placed strategically over the head, thereby inducing a current through the brain. In part, this serves as a modern alternative to the much older (1938) use of applied currents to treat depression, namely **ElectroConvulsive Therapy** (ECT), wherein pulses or sinusoidal voltages are applied to the scalp through electrodes, producing power levels of several hundreds of watts directly into the brain.

Another purely inductive device, **Pulsed Magnetic Field** therapy (PMF), has found great success in treating bony nonunions, a rather common problem in which fractures do not knit properly. This device was introduced by Bassett and Pilla[38] following a long history showing that living bone enjoys remarkable electric properties[39] that can be used to advantage in growth and repair processes[40]. In a very real sense, the PMF work on bone in the 1970s was the springboard for the development 25 years later of rTMS.

Electromagnetically-induced hyperthermia (**Oncotherm**)[41] and **Electrochemical Treatment** (EChT)[42] have both been found useful in treating late-stage cancers, the former mostly in Europe and Asia, and the latter in China. The Oncotherm device applies carefully directed radiofrequency devices to tumor sites, slightly elevating the local temperature, which has the interesting effect of killing off cancer cells without affecting healthy tissues. Neither procedure has as yet been approved by the FDA.

A much older device, dating back to the 1930s, **Diapulse**, applies radiant Joule heat deep into tissues. Because this device was introduced prior to the establishment of the FDA, its acceptance was ”grandfathered”, that is, allowed to be advertised and marketed on the basis of earlier widespread use. Electromagnetic energy is directed to specific areas of the body in the form of 600 pulses/s with each pulse lasting 65 ms. Although it was originally used to provide pain relief the extent of the therapeutic claims now includes “neurologically associated problems”. Along with a number of other devices making therapeutic claims related to radiofrequency use, the prominent frequency employed was 27.15 MHz, which has no special biological qualities, but is merely a frequency of choice permitted by the Federal Communications Commission (FCC).

This 27.15 MHz frequency has also appeared as the carrier wave in a similar arrangement to that used in the LEET insomnia device mentioned above, where one electrode is again placed in the mouth, in this case to treat cancer[43]. A much lower frequency, in the tens of Hz, modulates the 27.25 MHZ carrier. Presumably this ELF component represents the active anti-oncogenic component in this device.

Even higher frequencies, at 50 GHz and larger have also been reported as therapeutic aides. These devices, generally described as **Microwave Relaxation Therapy** (MRT)[44] machines are widely used in Russia and the Ukraine for mood behavior, and (anecdotally) to strengthen the immune system.

The author has previously attempted[45] to characterize neuroelectromagnetic therapies as falling into three categories: **subtle, gross, and disruptive**. The procedures of rTMS and ECT can be regarded as **disruptive**, considering that seizures have been associated with both, either deliberately or by accident. Similarly **gross** neurotherapies properly describe the great number of neural stimulators in use today. The term **subtle** is meant to convey the great difficulty in understanding how vanishingly small electric and magnetic signals are able to affect biological systems. It is abundantly clear that such signals cannot be the result of either Faraday induction of voltage or thermal changes due to Joule heating.

III. NON-INDUCTIVE NON-THERMAL MEDICAL APPLICATIONS

The question of subtle electromagnetic effects in biology is not new. Observations indicating that minutely small electric currents, at levels far weaker than allowed by simple energetic estimates, are capable of profound biological effects. These were first reported in connection with living bone. Electret applications[46], likely supplying no more than a few hundred nanoAmperes, were found to significantly affect growth rates in bone. This fact was subsequently used in a number of orthopedic devices operating at 1-2 mA to repair bony non-unions[47]. The great advantage of the PMF techniques mentioned above was that currents at this level could be introduced at the repair site in a completely non-invasive way.

More recently, the FDA-approved application of ion cyclotron resonance magnetic fields to the problem of bone repair[48] has all but replaced the use of both weak electric currents and PMF pulses. Magnetic fields from a portable coil tuned jointly to Ca^{2+} and Mg^{2+} are applied for 30 minutes a day over a period of weeks. It should be emphasized that the efficacy of this application, achieving repair rates of 70% or more, remains unexplained, except insofar as one considers ion cyclotron resonance phenomena as empirically factual.

Adey also recognized the fact that such signals caused effects that were not readily explained. In attempting to understand results obtained in his laboratory showing a distinctly nonlinear response in connection with the calcium-efflux experiments, he suggested that low-energy transmission occurs at cell membranes by means of solitonic waves[49].

The results listed in Table 1 for effects related to ELF magnetic fields have their counterparts in experiments conducted with AC electric fields. In some ways these are unexpected. Unlike the transparency of biological matter to low-frequency magnetic fields polarization effects in the extracellular medium and the large electric field at the cell membrane make it difficult to apply AC electric fields to cells. Some of the weak AC electric-field clinical approaches involve the use of invasive electrodes. Nonetheless these are noteworthy, considering the poor prognoses attached to illnesses such as glioblastoma.

Thus, one recent very promising therapy entails the use of electric fields at frequencies equal to or less than hundreds of kHz (**Tumor-Treating Fields**, or TTF) to treat aggressive glioblastoma and lung cancer[50,51]. Low-intensity electric fields, on the order of 1-2 V/cm, are found to slow the proliferation of all cells, cancer cells included. This is particularly advantageous in the treatment of brain cancer, because healthy brain cells tend not to proliferate in any case. Therefore the application of such fields is effective in slowing the increases in cancer cell production while leaving healthy cells unaffected. A somewhat similar effect has been discovered, but for applications at 50 Hz instead of hundreds of kHz. In this approach[52], a weak applied AC electric field is also used to fight cancer, not by reducing the proliferation of cancer cells, but by reducing their resistance to multidrug chemotherapy.

It is important to point out that these findings on the effectiveness of AC electric fields on cancer cell proliferation help illuminate why possible similar results that might be obtained using magnetic fields are so interesting. For one thing, there are problems related to AC electric field polarization effects that add constraints on how the cells are stimulated. By contrast because of tissue transparency to ELF magnetic fields, their clinical use will not only always be non-invasive, but also capable of being applied in more general ways.

Comparable effects of the sort observed using AC electric fields have already been observed using weak ELF magnetic fields. A number of reports have found changes in cell proliferation[8], particularly in lymphocytes, as a result of weak magnetic field stimulation. Further, in direct contrast to the electric-field reduction in chemotherapeutic resistance Liburdy discovered[53] that the resistance of breast cancer cells to tamoxifen was increased using 60 Hz magnetic fields.

Two interesting reports by Novikov highlight the clinical potential of weak magnetic fields. In the first case[54] he found that Ehrlich ascites cancer in rats can be dramatically reduced through the use of combined, ostensibly cyclotron-resonance tuned magnetic fields. In the second case[55] he demonstrated that these fields can also be used to hydrolyze, that is, break down, polypeptides by merely tuning to the charge-to-mass ratios of the constituent amino acids. One obvious clinical direction suggested by this work is to use this approach to break down the b-amyloid plaque protein associated with Alzheimer's disease. Experiments have indicated that this is indeed possible in animal models, but it is not yet clear if this plaque is a cause of this disease or simply one of its symptoms.

The last entry in Table 1 indicating that weak ELF magnetic fields can play an important role in stem cell applications[16] is particularly exciting. The most difficult aspect to treating heart failure is the inability of damaged heart muscle to regenerate, leading when possible to heart transplants. Stem cell regeneration of heart tissue is an obvious remedy to this problem but the results to date have in general been slow. This stalemate has been dramatically changed through the use of weak ICR magnetic fields. It was demonstrated that cardiac stem cells from humans when exposed for five days to ELF resonance fields tuned to Ca^{2+} enjoyed significantly greater proliferation and differentiation, perhaps paving the way for a minimally manipulative means of regenerating diseased hearts. Because of this result there is now heightened interest in the use of ELF magnetic fields to enhance the implementation of regenerative medicine and tissue engineering.

A very different approach to ICR medical therapy is found in the **Seqex** device[56] which applies an oscillating magnetic field to the patient's entire body while simultaneously taking advantage of the local parallel vertical component of the earth's magnetic field to achieve resonance. Its most celebrated use has been to treat the debilitating depression that often accompanies chemotherapy following cancer remediation[57], but there have also been numerous anecdotal reports claiming success in treating other diseases, for example multiple sclerosis. There is reason to believe that the efficacy of this device may be related to its dramatic effect on antioxidants. In addition to the fact that this device employs holistic application of the combined fields, it is unique in that the applied ICR frequency is not calculated from ionic charge-to-mass ratios, but is determined by first finding in a prior separate evaluation the specific frequency conditions that sharply alters the whole-body bioimpedance. Once determined this frequency information is stored on a "smart card" for future treatments on that patient. It is worth noting that the change in whole-body bioimpedance at resonance is consistent with the sharp changes in ionic conductivity that were observed by Zhadin and others. This device has not as yet been introduced into the United States for clinical evaluation.

IV. WELLNESS AND ILLNESS: THE ELECTROMAGNETIC PERSPECTIVE

The medical community continues to regard therapeutic regimens based on weak magnetic fields with great suspicion. This fact is best illustrated by contrasting the interest shown in the use of AC electric fields to treat cancer while similar results using magnetic fields have all but been ignored. We do not seek to diminish the potential importance of these electric field effects, but it is apparent that ELF magnetic field research is still thought of as too far outside the mainstream. One useful rationalization in trying to explain the AC electric field effects has been to implicate voltage-dependent ion channels as the key interaction site. This allows one to avoid the thorny question surrounding the intrinsic difficulty in the lack of penetration of AC electric fields into the cell. By contrast, even though there appears to be no such thing as magnetically responsive ion channels, ELF magnetic fields are not impeded by the large electric field of the cell membrane, reaching all compartments inside the cell equally.

One alternate view, when looking at electromagnetic effects, may be to regard a common parameter found in both the electric and magnetic cases, perhaps involving frequency or some function of frequency, as the key distinction. This has already been hinted at in connection with ICR biological interactions.

Recently the author and colleagues[26] advanced a radical new view of electromagnetic effects in biology, suggesting that these strange new electromagnetic interactions can be explained in terms of an endogenously available substrate resonantly coupled to biological ions that enables information transfer for purposes of regulation. In this approach the tweaking of biological systems with weakly energetic electromagnetic signals reveals an underlying order to organisms, one in which the electromagnetic is elevated above the biochemical.

However, even if this generalized concept of systemic electromagnetic wellness is correct, there still remains unexplained the molecular basis that might tell us why nanoAmpere currents can help initiate bone formation or why nanoTesla magnetic fields can hydrolyze proteins. These fully replicated observations are well outside the simplistic electrical engineering that is so often used to discuss such effects. For example, it is inappropriate to express this work in terms of

Specific Absorption Ratio (SAR), because a different yardstick is required. The low levels of power absorbed by the biological system are literally many orders of magnitude below the 1 Watt/kg prescribed as safe. We know that very low levels of electromagnetic can affect biological systems, but do not know how this happens. One clearly obvious truth yet to be generally accepted, yet of vital importance to everyone, is that these effects are profoundly quantum mechanical in nature[17-21], and have little connection to the traditional safety limitations imposed by electrical engineers.

V. CONCLUSIONS

There can be little doubt that weakly energetic electromagnetic fields are biologically interactive to the point where they can be usefully applied in medically relevant therapeutic procedures. Not only does this fact suggest a bright future for the role of electromagnetism in medicine, but it also underscores the need to be very cautious when examining the effects of low-level electromagnetic fields on people. This conclusion, slightly rephrased, was expressed by the author when he wrote[58]:

> ***In the long run, [weak-field exposures for medical purposes] may be the only way to prove the case for biological plausibility among those who presently choose to deny that weak field low frequency magnetic fields do indeed interact with biological systems.***

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SECTION 18

Electromagnetic Field Exposure Effects (ELF-EMF and RFR) on Fertility and Reproduction

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I. INTRODUCTION

Electromagnetic fields and radiofrequency radiation (RFR) interact with human tissues and may have adverse effects on fertility and reproduction. This review presents evidence for ELF-EMF and RFR effects on many parameters of male sperm function; leading to questions about the genotoxicity and carcinogenicity of such exposures on fertility and reproduction in men. Much of the evidence comes from human and animal studies on sperm and male fertility factors, but there are also studies showing adverse effects on fertility and miscarriage in women.

During the last four decades or so there has been a growing concern on the effects of electromagnetic radiations on biological systems in general. This is because of the global introduction of electronic devices on a massive level for communications and data transmission, personal wireless devices, air surveillance systems, industry applications, medical/diagnostic and therapeutic purposes that are now new sources of electromagnetic fields (ELF-EMF) and radiofrequency microwave radiation (RFR). This has added another layer of pollutant (electropollution) to a growing list of environmental contaminants in air, water, soil and from noise pollution which can adversely affect human health.

There are many sources of EMF in our environment and this non-ionizing radiation interacts with the human body. Use of electronic household items and cell phones are reported to decrease fertility potential in men by decreasing sperm count, motility, viability, inducing pathological changes in sperm and testes morphology, and so on (Erogul et al. 2006). In accordance with this, several authors (Agarwal et al. 2008, 2009; Kumar et al. 2010, 2011a; Pourlis 2009; Kesari et al. 2010, 2011, 2012) focused mainly on the male reproduction patterns. It involves the development from undifferentiated diploid stem cells to highly differentiated haploid stem cells. Spermatogenesis is a complex process and it is influenced by many genes and hormones. It takes place in the testis, which may be exposed to various microwave frequencies which are currently in use (Behari and Kesari 2006). Among various factors of infertility, oxidative stress has become the main focus of interest as a potential cause of male infertility (Agarwal and Said 2003; Aitken and Roman, 2008; Kumar et al, 2010, 2011a). Male infertility is commonly associated with high rates of DNA (deoxyribonucleic acid) damage in the spermatozoa and such damage is correlated with a wide range of adverse clinical outcomes. Several studies, especially at power frequency 50/60

Hz magnetic field have found an association of exposure to human health, with emphasis on a range of clinical conditions including childhood leukaemia, brain tumours, genotoxicity and neurodegenerative disease, infertility, birth defects, increased risk of miscarriage, childhood morbidity and de novo mutations (Hardell and Sage 2008; Gharagozloo and Aitken 2011; Garcia et al. 2008; Huss et al. 2008; O'Carroll and Henshaw 2008; International Agency for Research on Cancer (IARC) Monographs of the Evaluation of Carcinogenic Risks to Human 2002; California Health Department Services (CHDS) Report 2002). Sperm DNA damage is therefore regarded as a potential risk factor to the development of normal human embryos leading to impaired embryonic development.

II. THE BIOPHYSICS OF EXTREMELY LOW FREQUENCY FIELDS

Whenever a body having finite conductivity (biological body) is intercepted by EMF it induces electric fields and circulating electric currents, which in turn competes with endogenous current and voltages, thus disturbing normal physiological balance. The depth of penetration within the body depends upon its frequency and the electric properties of the exposed portion in the body. If the current density exceeds a certain threshold value, excitation of muscles and nerves due to membrane depolarization is possible. The mode of interaction of non-ionizing radiation with biological systems can be broadly divided into two parts: extremely low frequency and radiofrequency/microwaves.

Whenever an electric field interacts with a biological body the incident field will be distorted, such that the external field will be nearly perpendicular to the boundary surface. At 60 Hz

$E_{internal} / E_{external} \approx 4(10^{-8})$. (1)

Thus a 60 Hz external field of 100 kV/m will produce an average internal E field of the order of 4mV/m.

As far as the magnetic components of the extremely low frequency fields are concerned, magnetic permeability μ of most biological materials is practically equal to that of free space $(4\pi.10^{-7})$ H/m. This signifies that ELF H field 'inside' will be practically equal to the H field 'outside'. Only exceptions could be those biological materials that have magnetic particles inside. A time varying magnetic field (also electric field) can also induce electric currents into stationary conducting objects. Thus, all modes of interaction of time varying E fields with living matter may be triggered by time-varying (not by static) magnetic field. According to Faraday's law of electromagnetic induction time varying magnetic flux will induce E fields with resulting electrical potential differences and "eddy" currents through available conducting paths. Sources generating low frequency electric and magnetic fields are more likely to produce physiologically significant internal E fields through the mechanism of magnetic induction. If an erect person is targeted by a vertical electric field it will be considerably “enhanced” at the top of the person’s head and shoulder, and one would predict therefore that the field in the tissue would also be enhanced above that of a flat slice exposed to the same field (Deon, 1982). In a 60 Hz electric field of 1kV/m in air, the current densities (Am/m^2) in neck, waist and ankle turn out to be $0.591x10^{-3,}$ $0.427\ x^{-3}$ and $3.35x10^{-3}$ respectively (Polk 1986).

III. THE BIOPHYSICS OF RADIOFREQUENCY AND MICROWAVE FIELDS

The biological bodies are inhomogeneous, having tissue-specific dielectric properties and the complexity of the shape; which make the computations of the induced field difficult. The fields induced inside the body act differently depending upon the frequency and more particularly on (L/λ), (where L is the length of the biological body and λ the wavelength of the incident field) upon, but are not limited to the following parameters:

(i) The location of the field with respect to the surroundings, e.g. if there are metallic objects around, the person is grounded or otherwise.
(ii) Polarisation of the incident wave with respect to the orientation of the human body.
(iii) Size of the human body (L) with respect to the wavelength (λ) of the incident radiations (L/λ).
(iv) The portion of the human body.
(v) The electrical properties of the tissue in question.

In free space propagation of electromagnetic field the power density is given by

$$\text{Power density} = E^2/1200\,\Pi \ \text{mW/cm}^2 \qquad (1)$$

Where, E is the electric field strength.

The frequency in the radio frequency-microwave region are somewhat penetrated inside the biological body interacting with the tissues inside.

From simple biophysical considerations, it follows that each body has a characteristic resonant frequency depending upon the length of the long axis. Correspondingly, for the same level of incident exposure the average value of power absorbed is dependent upon the length of the body, the degree of decoupling decreasing the average value of SAR by more than an order of magnitude. It is suggestive that absorbed RF energy can be converted into other form of energy and can cause interference with the functioning of the biological systems. A significant portion of this energy is converted into heat (absorption). The biological effects are frequency dependent. Well below 100 KHz, the induced fields can even stimulate nervous tissue.

IV. FERTILITY AND REPRODUCTION EFFECTS: ELF-EMF FIELD EXPOSURE

Since the biological body is diamagnetic it is transparent to the static magnetic field. It can therefore interact with the motional activity of paramagnetic materials. Amara et al (2006) has shown that adult male rats exposed to such fields (128 mT, 1hr/day for 30 days) show a decrease in testosterone levels and induced DNA oxidation. Subchronic exposure failed to alter spermatogenesis in rat testis. In a similar study Hong et al (2005) also concluded that 50 Hz EMFs (0.2 mT or 6.4 mT, exposed for a period of 4 weeks) may have the potential to induce DNA strand breakage in testicular cells and sperm chromatin condensation in mice.

Al-Akhras et al (2006) also treated male adult rats to 50 Hz sinusoidal magnetic field (25μT or 250 mg) for 18 consecutive weeks. They reported no significant effects on the absolute body weight and the weight of the testis of the exposed rats. However the weight of the seminal vesicles and preputial glands were significantly reduced in the exposed male rats, along with significant reduction in sperm count of the exposed rats. There was no significant effect on the serum levels of male follicle stimulating hormone (FSH) during the 18 weeks of exposure period. On the other hand there was a significant increase in the serum levels of male luteinizing hormone (LH) after 18 weeks of exposure ($p<0.005$) while testosterone levels were significantly decreased after 18 weeks of exposure period. These results suggest that long term exposure of ELF could have adverse effects on mammalian fertility and reproduction.

Different results have been presented by Chung et al (2005) where animals exposed in-utero and subsequent neonatal exposure to a 60 Hz EMF(field strength 500 μT or 5000 mG) from day 6 of gestation to day 21 of lactation, did not produce any detectable alteration in offspring spermatogenesis and fertility.

Akdag et al (2006) examined the effects of ELF magnetic fields (1.35 mT) on sperm count, malondialdehyde concentration, the histology of organs as: testes, brain, liver, and kidney tissues, p53 immunoreactivity of bone marrow and the serum concentrations of Cu^{2+}, Zn^{2+},Mn^{2+} and Fe^{3+} in rats. These authors found no statistically significant alteration except in Mn^{2+} concentrations ($p<0.001$).

Influence of ultrasound (frequency 2,4 and 8 MHz) and constant magnetic field (7T) on gametes, zygotes and embryos of the sea urchin were studied by Drozdov et al (2008). Magnetic field exposure interrupts the process of the gamete fusion but did not influence gametes, embryos, or embryonic development. The nature of these two stimuli is of different type. Ultrasound may heat up the water if is of sufficient power, by way of increase in water temperature and cavitation temperature, which may also break the cellular structure. The effect of magnetic field is connected to the response of the cortical cytoskeleton, which consists of bundles of actin microfilaments. The rearrangement of the cortical cytoskeleton occurs during the first 20 minutes after the contact of sperm with the egg.

Kim et al (2009) examined the effect of a 16-week continuous exposure to ELF magnetic field (MF) of 14 or 200 μT (140 or 2000 mG) on testicular germ cell apoptosis in mice. They reported no significant adverse effects of MF on body weight and testosterone levels in mice. In TUNEL staining (in situ terminal deoxynucleotidyl transferase-mediated deoxy-UTP nick end labelling), germ cells show a significantly higher apoptotic rate in exposed mice than in sham controls ($P<0.001$). TUNEL-positive cells were mainly spermatogonia. In an electron microscope study, degenerating spermatogonia showed condensation of nuclear chromatin similar to apoptosis. These results indicate that apoptosis may be induced in spermatogenic cells in mice by continuous exposure to 60 Hz of 14 MF μT (140 mG).

Roychoudhury et al (2009) examined the effects of 50 Hz extremely low frequency electromagnetic field on in vitro rabbit spermatozoa motility. These authors also studied the effects after insemination. Pooled semen samples and a control were exposed to 50 Hz ELF EMF. The difference of the test groups G1 and G2 with the control group CG (75.56%) for spermatozoa motility were found to be significant ($P<0.01$). Differences were significant ($P<0.01$) for curvilinear velocity (VCL) between the test group G3 (122.38 μ/s). Hormonally simulated adult (9-12 months) females (n=140) were inseminated with semen samples from G1, G2, G3 and G4 (0.88×10^9 spermatozoa /0.5 ml average insemination portion) immediately after ELF EMF exposure and fertilization (kindling) rates were calculated. For the G2 it was 54.28% data indicate 50 Hz ELF EMF induced alterations of spermatozoa motility and kindling rate in rabbits, therefore influencing fertility.

Cao et al (2009) also reported that magnetic fields at 1000 Hz or 2000 Hz may damage the testis by inducing injury to seminiferous tubules and Leydig cells, thickening the basal membrane, derangement, exfoliation, massive apoptosis and necrosis of spermatogenic cells in the lumen, epididymis, and consequently result in the absence of sperm.

Bernabo et al (2010) assessed the effect of acute (1hr) exposure of boar spermatozoa to an extremely low frequency electromagnetic field (ELF-EMF) (50 Hz, MF 0-2 mT) on early fertility outcome. They examined morpho-functional integrity of capacitated spermatozoa in vitro and reported in vitro ELF-EMF >0.5 mT induced a progressive acrosome damage, thus compromising the ability of spermatozoa to undergo acrosomal reaction after zona-pellucida stimulation and reducing the in vitro fertilization outcome. These effects became evident at 0.75 mT and reached the plateau at 1 mT. Under in vivo conditions, ELF-EMF intensity of 1 mT was able to compromise sperm function, significantly reducing the fertilization rate. In addition, the exposure of oviducts field ≥0.75 mT in the absence of spermatozoa was able to negatively affect early embryo development. In fact it was found to cause a slowdown in the embryo cleavage. It is apparent that at mentioned intensities the fields has negative effect on early fertility outcome in a predictive animal model.

Earlier these authors (Bernabo et al 2007) reported that MF-ELF influence negatively by dramatically effecting sperm morphology and function.

The blood-testis barrier is sensitive to environmental stimulation, which can affect its permeability and then result in antisperm antibody (AsAb) generation, which is a key step in male immune fertility. Wang et al (2010) reported the results of male mice exposed to electromagnetic pulse (EMP) by measuring the expression of tight-junction of associated proteins(ZO-1 and Occludin), vimentin microfilaments, and mice were sham exposed or exposed to EMP at two different intensities (200 kV/m and 400 kV/m) for 200 pulses. The testes were collected at different points after EMP exposure. Immunofluorescence histochemistry, western blot, laser confocal microscopy and RT-PCR were used in this study. Compared with sham group, the expression of ZO-1 and TGF-beta3 were significantly decreased accompanied with unevenly stained vimentin microfilaments and increased serum AsAb levels in EMP-exposed mice. These results are indicative of a potential BTB injury and immune infertility in male mice exposed to certain intensity of EMP.

Lorio et al (2011) studied the functional relationship between the energy metabolism and the enhancement of human sperm motility induced by ELF-EMF was investigated. Sperm exposure to ELF-EMF resulted in a progressive and significant increase of mitochondrial membrane potential and levels of ATP, ADP, and NAD(+) associated with sperm kinetic parameters. However no significant effects were detected on other parameters such as ATP/ADP ratio and energy change. When carbamoyl cyanide m-chlorophenyllhydrazone (CICCP) was applied to inhibit the oxidative phosphorylation in the mitochondria, the values of energy parameters and motility in the sperm incubated in the presence of glucose and exposed ELF-EMF did not change, thus indicating that the glycolysis was not involved in mediating ELF-EMF stimulatory effect on motility. By contrast, when pyruvate and lactate were provided instead of glucose, the energy status and motility increased significantly in ELF-EMF-treated sperm. Under these culture conditions, the inhibition of glycolytic metabolism by 2-deoxy-D-glucose (DOG) again resulted in increased values of energy and kinematic parameters, indicating that gluconeogenesis was not involved in producing glucose for use in glycolysis. These authors concluded that the key role in mediating the stimulatory effects exerted by ELF-EMF on human sperm motility is played by mitochondrial oxidative phosphorylation rather than glycolysis. Earlier these authors (Lorio et al 2007) reported that ELF-EMF exposure can improve spermatozoa motility and that this effect depends on the field characteristics. ELF-EMF with 50 Hz and square wave shape (amplitude 5 mT),while that of a sine wave of the same amplitude (also of 2.5 mT) and the same frequency had no such effect. Further a three hour exposure in the first case had the effect on sperm motility persisting for 21 hours.

People connected to local area networks wirelessly (Wi-Fi) were examined for human spermatozoa. These authors (Avendano et al 2012) selected sperms from 29 healthy donors for their capability to swim. This study using a laptop as a source contributed both ELF-EMF and RFR to the exposure conditions. Each sperm suspension was divided into two aliquots. One sperm aliquot (experimental) from each patient was exposed to an internet connected lap top by Wi-Fi for 4 hours, whereas the second aliquot (unexposed) was used as control and incubated under identical conditions without being exposed to the laptop. These authors evaluated sperm motility, viability, and DNA. These authors reported that normozoospermic, exposed ex vivo during 4 hour to a wireless internet –connected laptop showed a significant decrease in progressive sperm motility and an increase in DNA fragmentation. Level of dead sperm showed no significant differences between the two groups. They concluded that the effect (which is non-thermal) decreased motility and induced DNA fragmentation. It is therefore speculated that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility.

Sage et al (2007) reported that personal and occupational use of personal digital assistants (PDAs or palm-held wireless units) produce high intensity bursts of ELF-EMF exposure in persons that carry a PDA close to the body (i.e., in a pocket or in a belt); or held to the head for cell phone conversations. ELF-EMF emissions of 10µT (100 mG) were recorded on PDAs during normal office use over a 24 hr test period. Results of ELF-EMF measurements show that email transmit and receive functions produce rapid, short duration ELF-EMF spikes in the 2-10µT (20 to 100 mG) range, each lasting several seconds to over a minute, depending on the download size. Switching the PDAs produced continuously elevated ELF-EMF pulses of over 90 µT on two units. Thus the user who wears the PDA may be receiving high-intensity ELF-EMF pulses throughout the day and night.

Avendano et al (2012) investigated the effect of laptop computers connected to internet through Wi-Fi on human sperm motility. Donor sperm samples, mostly normozoospermic, exposed ex vivo during 4 hours connection showed a significant decrease in progressive sperm motility and an increase in sperm DNA fragmentation due to nonthermal effect, thus showing potential risks to male fertility.

Bellieni et al (2012) has investigated a much wider issue of reproduction relating to that of fetal growth and the effect of emissions from lap top computers (LTC). Such wireless and ELF-EMF exposures may have adverse effects on the offspring. They measured magnetic field in the range 1 Hz -400 kHz range as emitted from LTC. These field have the advantage that being quasi static can penetrate inside the body and thereby induce voltage and induce currents. The authors reported that the magnetic field at dominant frequencies ranged from 1.8-6 µT (18 to 60 mG), where from the power supply ranges from 0.7 to 29.5 µT (7 to 295 mG). They found that the power supply produces strong intracorporal electric current in the fetus and in the mother, higher than ICNIRP (1998) basic restriction recommend to prevent adverse health effects. The field emissions from video terminals are reported to be low (0.1µT or 1 mG) and the effect of higher exposures needs to be investigated (Bellieni et al 2012)

Sun et al. (2005) investigated the effects of EMR emitted by computers on human sperm quality and did not find any adverse effect.
An observation that women who use video display terminals suffers miscarriages has led to the beginning of diagnosing the possible adverse effects of electric and magnetic fields

Extremely low frequency electromagnetic fields are likely to produce greater damage to the body systems for several reasons. One that these frequencies are close to those of physiological range and hence any overlap of these can perturb on-going biological processes. When in close contact with the body the generation of eddy currents and accompanied heating are added parameters. To differentiate their respective contributions on biological system is an impossible demand.

Extremely low frequency EMF effects induced due to electric(E) blankets generate eddy currents in the body.60 Hz magnetic field exposure generate about 3-4 mG for waterbeds (W) and about 15 mG for E (Electric Blankets),as reported by (Wertheimer and Leeper 1986). They have estimated that electric fields are of the magnitude 100 V/m. E and W both have the potential for providing excessive body heating, which may have adverse effect on sperm (Van Demark and Free 1970), leading to adverse effect on the process of embryogenesis (Edwards et al 1974,Lacy et al 1981). This high temperature could also be teratogenic in humans too (Miller et al 1978, Fraser and Skelton 1978).It is obvious that either the heat or the electromagnetic fields produced by electric or bed heating might affect the fetus. These authors concluded that E or W use has a direct effect on fetal development. It is argued that heat or electromagnetic field exposure is he seasonal. Both prolonged gestation and fetal loss have been shown to be associated with high blanket settings used by the mother, but not those used by the father. Earlier workers have also pointed out that electromagnetic exposure may cause abnormal fetal development (Delgado et al 1982).Marx (1981) pointed out that current and field distribution in embryos, responsible for normal fetal development are disturbed due to the presence of externally imposed fields .

Li et al (1995) studied the effect of prenatal electromagnetic field exposure on the risk of congenital urinary tract anomalies (CUTAs) among women with a history of subfertility as well as in general population. These authors found no consistent relation between the risk of CUTAs and prenatal exposure to electromagnetic fields from E,W ,and video display terminals among all cases of controls. The risk appeared to increase with increasing duration of use and was greatest among women who used Es during the first trimester .CUTA cases exposed to Es prenatally appeared more likely to have anomalies of the ureter, bladder than unexposed cases. However there is an absence of association with the risk of electrically heated water beds and video display terminals and demands further investigations. They further pointed out that only women with a history of subfertility were subject to said exposure ,since the positive association between potential E use and risk of CUTAs was observed in this group. They concluded that out of the three E,W and video terminals, E has the maximum capacity,keeping in view the proximity with all parts of the body and duration of exposure. Women with subfertility history are more prone to adverse pregnancy outcome.

Juutilainen et al (1993) carried out case control study, although on a small number ,on women .They measured magnetic field at the front door and reported a five-fold increase in preclinical miscarriage. Lee et al (2001) conducted a case control study nested in a miscarriage study. They defined cases as women who had a clinical miscarriage before 20 weeks of gestation and controls as women who had a live birth. They observed a gradient in miscarriage risk as the number of environmental parameters increased above the 50th percentile. Their findings are not consistent with the results of mechanistic and mammalian studies (Portiere and Wolfe 1987) ,while some laboratory results supports alterations in the development of chick embryos exposed to EMF.(Farrell et al 1997). While numerous data have been generated but are inconclusive and the possibility of more funding seems remote.

In summary the possibility of immediate abortion has not found favour with the researchers. However a weak link is possible. A temperature rise causing adverse effect on sperm is possible and certainly avoidance is recommended more so for pregnant women. Another point of interest would be to see if any adverse effects are reversible.

The area certainly demands more investigations.

A summary of these data is presented in Table 1 (Studies on Effects of ELF-EMF on Fertility and Reproduction).

Table 1: Table showing the overall Effect of Extremely Low frequency electromagnetic field effects on reproduction and fertility

Organism used	**Mode of exposure**	**Parameters studied**	**Conclusion**	**Reference**
Human sperm	internet-connected laptop by Wi-Fi for 4 hours	sperm motility and an DNA fragmentation	Decrease in motility and increase in DNA fragmentation	Avendano et al, 2012
Human sperm	ELF -EMF	Sperm kinematics	Increase in mitochondrial membrane potential	Lorio et al 2011
Mice	4h d 2 m at 3 mT EMF with Polygonum aviculare	Sperm motility and morphology	Motility affected. With *P. aviculare* is sperm quality increased	Milan et al. 2011
Boar spermatozoa	Acute (1h) 50 Hz ELF	Early embryo development	Reduction in fertilization rate, Affect embryo development	Bernabo et al. 2010.
NMRI mice (Naval Medical Research Institute)	50 Hz, 0.5 mT EMF 4 h for 2 weeks	Fertility and height of epithelial cells	Decrease in blastocyte and increase in the height of epithelial cells	Rajaei et al.2010
Rabbit spermatozoa	50 Hz ELF	Spermatozoa motility	Change in motility and kindling rate	Roychoudhury et al.2009
ICR mice	X- ray, 1000 Hz and 2000Hz	Sperm motility	Affect testis function	Cao et al. 2009
BALB/c mice	ELF 60 Hz ,0.1 or 0.5 mT 14 or 200 mT	Apoptosis	Induced apoptosis	Kim et al. 2009
Balb C mice	Electromagnetic pulse (EMP)	Tight-junction-associated proteins,transforming growth factor-beta and AsAb level in serum	Decrease in expression of protein	Wang et al 2010

Table 1 continued …

human spermatozoa	ELF-EMF 5 mT and frequency of 50 Hz.	sperm motility	Square waveform of 5 mT amplitude and frequency of 50 Hz increase sperm motility.No change in 5 mT sine wave (50 Hz) and a 2.5 mT square wave (50 Hz	Lorio et al 2007
Sprague – Dawley rat	ELF 2hour for 2 months	Sperm count, histology, p53 immunoreactivity of bone marrow	No adverse effect. Increase in Mn2+.	Akdag et al 2006
Rat	static magnetic field (SMF) and cadmium	Antioxidant enzymes activity	SMF with Cd disrupt antioxidant response	Amara et al 2006
Mice	50 Hz .02,3.2or 6.4 mT for 2 weeks or 4 weeks	Testicular histology, weight quantity and motility of sperm	Reduced testicular weight, decreased sperm motility. High rate of deformity in sperm	Hong et al 2003
Pregnant women	Case control study (Magnetic field)	Miscarriage	Miscarriage before 20 weeks of gestation	Lee et al 2001
Sperm	12.5, 25, 50 and 100 cGy X-rays	DNA damage	Increase in DNA migration	Singh and Stephens 1998
Pregnant women	Electric blanket, electric heated water bed, and video display terminal	Congenital urinary tract abnormality(CUTA)	Increased risk of CUTA	Li et al 1995
Human	Extremely low frequency EMF(60Hz)	Abortion rate, Fetal development	Excess abortion	Wertheimer and Leeper(1986)

V. FERTILITY AND REPRODUCTION EFFECTS REPORTED FOR RADIO-FREQUENCY AND MICROWAVE EXPOSURE

Nakamura et al. (2000) found that exposure to 2.45 GHz continuous wave (CW) microwave at 2mW/cm^2 power density for 90 min decreased uteroplacental blood flow, increased progesterone and PGF$_2\alpha$ in pregnant rats. Dasdag et al. (2003) reported the decrease in seminiferous tubule diameter in male rat testes after exposure. They used commercially available 890-915 MHz GSM (global signal module) with 0.141 W/kg whole body SAR. More recently, Aitken et al. (2005) found significant damage to mitochondrial and nuclear genome in epididymal spermatozoa of mice, when exposed to RF 900 MHz EMW, 12 hr a day for 7 days. Several authors (Fejes et al. 2005; Ji-Geng et al. 2007; Kesari and Behari, 2008) have also observed that carrying the mobile phones near reproductive organs for longer time may have negative effects on the sperm motility and male fertility.

Aitken et al (2005) exposed mice to 900 MHz radiofrequency electromagnetic radiation at a SAR of 90 mW/kg inside a waveguide for 7 days (12 hr/day). Following exposure DNA damage to caudal epididymal spermatozoa was assessed. These authors reported no gross evidence of single-or double strand DNA breakage in spermatozoa taken from treated animals. However an analysis of DNA integrity revealed significant damage to both the mitochondrial genome ($P<0.05$) and the nuclear beta-globin locus ($P<0.01$). This study suggests that while RF EMR does not have a dramatic impact on male germ cell development, a significant genotoxic effect on epididymal spermatozoa is seen.

Kilgalton and Simmons (2005) report decreased semen quality with prolonged use of cell phones with negative effects on sperm motility characteristics (Fejes et al, 2005). It has been shown that sperm DNA damage is not repaired, because of chromatin structure (Singh and Stephens 1998).

Yan et al (2007) studied the effects of cellular phone emissions on sperm motility in rats. Rats were exposed to two 3-hr periods of daily cellular phone emissions for 18 weeks, sperm samples were then collected for evaluation. These authors concluded that exposed group of

rats exhibited a significantly higher incidence of sperm cell death than control group rats. In addition, abnormal clumping of sperm cells was present in rats exposed to cellular phone emissions and absent from control group rats. A study carried out in Poland (Wdowiak et al 2007) on the population using mobile phone (GSM equipment), spread over a period (1-2 years) indicates sperm quality is lowered. The authors report a decrease in the percentage of sperm cells with normal motility in the semen. The decrease in motility correlates with the frequency of using mobile phones. These two finding seem to be mutually supportive.
However there are also reports indicating no effects (Panagopoulos and Margaritis 2008, 2009, 2010).

Overall, the evidence from various laboratories studying fertility and reproduction effects over the last ten years is important enough raise questions about possible public health consequences of chronic, long-term exposure to mobile phone use, and when carried on the body close to the reproductive organs. While assessing the biological implications of mobile phone radiofrequency exposures, field based experiments are not possible. Sham exposure controls cannot be obtained. Therefore it is imperative to fall back upon laboratory experiments performed in a variety of situations (e.g. animals at different distances from the mobile phone and head) while also simulated variable distances and angles for the mobile phone variation while in actual use.
Gutschi et al (2011) studied human sperm obtained from 2110 patients attending clinics from 1993 to 2007. Semen analysis was performed in all patients. Serum free testosterone (T), follicle stimulating hormone (FSH), luteinising hormone (LH) and prolactin (PRL) were collected from all patients. Information on cell phone use from each patient was collected and the subjects were divided into two groups according to their cell phone use. Group A: cell phone use (n=991), Group B: no use (n=1119). Patients with cell phone use showed a significant higher T and lower LH levels than those who did not use a cell phone. However no significant difference was observed regarding FSH and PRL values. These authors concluded that cell phone use had a negative effect on sperm quality in men.
Kesari et al (2011) assessed free radical formation due to mobile phone exposure (2 hr a day for 35 days) and examined fertility patterns in 70-days old male Wistar rats. The specific absorption rate of the mobile phone was 0.9 W/kg. An analysis of anti-oxidant enzymes glutathione peroxidise($p<0.001$) and superoxide dismutase ($p<o.007$) showed a decline, while

an increase in catalase ($p<0.005$) was observed. Malondialdehyde ($p< 0.003$) showed an increase and histone kinase ($p=0.006$) showed a significant decrease in the exposed group. Correspondingly, micronuclei also showed a significant decrease ($p<0.002$). A change in sperm cell cycle of G_0 –G_1 ($p=0.42$) and G_2/M ($p=0.022$) was recorded. These authors concluded that changes occurred due to overproduction of ROS and oxidative damage, leading to infertility.

Yan et al (2007) studied the effects of cellular phone emissions on sperm motility in rats. Rats were exposed to two 3-hr periods of daily cellular phone emissions for 18 weeks. After the exposure period, sperm samples were collected for evaluation. The authors concluded that exposed group of rats exhibited a significantly higher incidence of sperm cell death than control group rats. In addition, abnormal clumping of sperm cells was present in rats exposed to cellular phone emissions and absent from control group rats.

A related issue is the corresponding effect on male infertility.

Sommer et al (2009) undertook a very exhaustive study where male and female mice were chronically exposed (life-long, 24 hr/day) to mobile phone frequency EMF at 1966 MHz (UMTS). They studied their development and fertility patterns over four generations by investigating histological, physiological, behavioural and reproductive functions. They tested SAR from the time of mating at 0 (sham), 0.08, 0.4 and 1.3 W/kg. Power densities were kept constant for each group (0, 1.35, 6.8 and 22 W/m^2), resulting in varying SARs due to different number of adults and pups. The results show no harmful effects of exposure on the fertility and development of the animals. The number and the development of the pups were not affected by the exposure. These authors concluded no harmful effects occurred with long-term exposure of mice to UMTS mobile phone frequency radiation over several generations.

DeIuliis et al (2009) used purified human spermatozoa for exposure to electromagnetic radiation at 1.8 GHz with specific absorption rates varying from 0.4 to 2.75 W/kg. These investigators reported that motility and vitality were significantly reduced after RFR exposure, while the mitochondrial generation of reactive oxygen species and DNA fragmentation was significantly elevated ($P<0.001$). They also found a highly significant relationship between SAR, the oxidative DNA damage biomarker 8-OH-dG, and DNA fragmentation after exposure. These results have bearing on safety of people of reproductive age, and wellbeing of their offspring. Erogul et al (2006) also support these finding by showing effect on sperm motility and that long-term exposure may lead to behavioural or structural changes of the male germ cell. These may appear later in life and need investigation on a longer term basis.

As a follow up of the above, Otitoloju et al (2010) exposed male mice to radiofrequency radiations at mobile phone (GSM) base station-level RFR. Sperm head abnormalities occurred in 39% to 46% of exposed mice, but in only 2% of the controls ($P<0.005$). The major abnormalities observed were knobbed hook, pin head and banana-shaped sperm head. The abnormalities were also found to be dose-dependent. This may have severe consequences for the off spring.

Gul et al (2009) investigated toxicity of microwaves (as emitted by cellular phones on ovaries in rats. In this study 82 female rats of aged 21 days (43 in the study group and 39 in the control group) were used. Pregnant rats exposed to mobile phones that were kept underneath the cages during the whole period of pregnancy. A mobile phone in a standby position for 11 hr and 45 min was turned on to speech position for 15 min every 12 hr and the battery was charged continuously. On the 21st day after the delivery , the female rat pups were killed and the right ovaries were removed. The volumes of the ovaries were measured and the number of follicles in every tenth section was counted. These authors found that the number of follicles in pups exposed to mobile phone microwaves suggest that intrauterine exposure has toxic effects on ovaries.

Salama et al (2010) examined the accumulating effects of exposure to electromagnetic radiation emitted by a conventional mobile phone (800 MHz, standby position, kept opposite to the testis) on the testicular function and structure. The animals were exposed 8 hr daily for a period of 12 weeks in a specially designed cage. Semen analysis and sperm function tests were conducted weekly. Other parameters examined were histological testicular sections and serum total testosterone. When compared with other two groups (stress control and ordinary), the exposed animals showed a drop in sperm concentration at week 6, which became significant at week 8. Mobile sperm population showed similarity amongst the three study groups until week 10 when it declined significantly, and thereafter in phone and stress control groups, with more significant decline in the exposed animals (50.6% and 72.4%, respectively). Histological examination showed a significant decrease in the diameter of seminiferous tubules in the exposed group vs the stress and ordinary controls (191 μm vs. 206 and 226 μm, respectively). The authors concluded that the pulsed radiofrequency emitted by a conventional mobile phone kept in the standby position could affect the testicular function and structure in the adult rabbit.

Falzone et al (2011) evaluated the effect of RF-EMF on sperm characteristics to assess the fertilizing potential of sperm. They exposed highly motile human spermatozoa to 900 MHz for an hour (SAR =2.0 W/kg) and examined effects at various time after exposure. The acrosome reaction was evaluated using flow cytometry. They did not find any effect on sperm propensity for the acrosome reaction. They obtained significant reduction in sperm head area (21.5±4% vs 35.5±11.4%) was obtained when compared among exposed and unexposed samples. Sperm zona binding was assessed directly after exposure. The mean number of zona-bound sperm of the test hemizona and controls was 22.8±12.4 and 31.8±12.8 ($p<0.05$) respectively. They concluded that though the radiation exposure did not adversely affect the acrosome reaction, it had a significant effect on sperm morphometry. They also observed a significant decrease in sperm binding to the hemizona. These data point toward sperm fertilization potential. These studies are in contradiction that fertility impairment was not caused by the induction of apoptosis in spermatozoa (Falzone et al 2010).

In a study undertaken by Ribeiro et al (2007), while experimenting with male Wistar rats, they exposed testis in the frequency and in the range of intensity (1835-1856 MHz, 0.04-1.4 mW/cm^2). The authors reported that the total body weight and absolute and relative testicular and epididymal weight did not change significantly, nor did the epididymal sperm count.

Human spermatozoa are known to be known to be vulnerable to oxidative stress because of abundant availability of substrates for free radical attack, and the lack of cytoplasmic space to accommodate antioxidant enzymes. The ROS generation does DNA damage, besides reducing fertility. The former has been linked with poor fertility, incidence of miscarriage and possible morbidity in the offspring, including childhood cancer.

There are other reports showing lack of effect on testicular function in experimental animals in the non-thermal range. They concluded that the responses are identical to those produced by hyperthermia caused by mere heating(Ribeiro et al 2007, Sommer et al 2009).

Comparison between non-modulated (DTX) and Modulated (Talk Signal) GSM Radiation

In an experimentation with insects, Panagopoulos (2011) divided these into two groups: a)the exposed (E) and b) the sham exposed (control) group (SE). Each of the two groups consisted of ten female and ten male newly emerged adult flies. The sham exposed groups had identical treatment as the exposed ones, except that the mobile phone during the "exposures" was turned off. The duration of exposure was 6 min per day in one dose extending over a period of 5 days.

In the first part of the exposure (1A) the insects were exposed in non-modulated GSM 900 MHz radiation (TDX-discontinuous transmission mode –signal) while in the second part (1B) they were exposed to modulated GSM 900 MHz radiation (or GSM talk signal). In both cases, the exposures were performed with the antenna of the mobile phone in contact with the walls of the glass vials containing the insects.

The difference between the modulated and the corresponding non-modulated GSM radiation is that the intensity of the modulated radiation is about ten times higher than the intensity of the corresponding non-modulated from the same handset (mobile phone) and additionally that the modulated radiation includes more and larger variations in its intensity within the same time interval, than the corresponding non-modulated one (Panagopoulos and Margaritis 2008). The power level of exposure for the modulated signal was 0.436±0.060 mW/cm^2 and the corresponding mean value for the non-modulated emission was (0.041±0.006) mW/cm^2. The measured ELF mean values of electric field intensity of the GSM signals excluding the ambient fields of 50 Hz were 6.05±1.02 V/m for modulated signal and 3.18±1.10 V/m for the non-modulated signal.

Experiments with the non-modulated GSM 900 MHz radiation (non-speaking mode of transmission) showed that this radiation decreased insect reproduction by an average of 18.24%. Correspondingly experiments with modulated GSM at 900 MHz (GSM "talk" signal) exposure shows that the radiation decreases reproduction by an average of 53.01 %. Above results indicate that the decrease in population is linked with intensity of the radiation. These authors concluded that between 900 MHz and 1800 MHz, the former is more bioactive owing to the difference in radiation intensity. Performing experiments at various distances (0 to 100cm) from mobile phone, Panagopoulos (2011) reported that the distance dependence is not linear. At the distances at 0 and 30 cm (intensity 378 µW/cm^2 and 10 µW/cm^2 respectively) show a maximum of decrease in reproductive capacity (window of maximum bioactivity). Correspondingly for GSM 1800 MHz at 0 and 20 cm (intensity 252µW/cm^2 and 11µW/cm^2 respectively) bioactivity is maximum (decrease in reproduction, window of maximum bioactivity) i.e. in the vicinity of free space wavelength of the corresponding radiation. For distances greater than 20 cm (up to 80 cm) the effect decreases rapidly and becomes very small for distances longer than 40 cm, but it is still evident for distances up to 80 cm (intensity down to 1.1µW/^{2}). These authors have further pointed out that it is the intensity which is primarily important rather than the frequency or the distance as such.

These distances (30 and 20 cm from GSM 900 MHz and GSM 1800 MHz correspond to the same RF intensity (10μW/cm^2) and also to the same electric field intensity of about 0.6-0.7 V/m. Maximum bioactivity is attributed to a distance of 0 cm or at approximately the two nodes of the wavelength, after which the effect declines. These authors reported no temperature increase inside any of the vials. They further concluded that the ELF components of digital mobile telephony signals that play a key role in their bioactivity, alone or in combination with the RF carrier signal . This also suggest that low frequency signals are more bioactive than higher frequency ones. Accordingly, electric field of the order of 10^{-3} V/m are able to disrupt cell function, perhaps by irregular gating of electrosensitive ion channels on the cell membranes. We conclude that both the GSM signal at 900 MHz and 1800 MHz fields appear to possess sufficient intensity for this for distances up to 50 cm from the antenna of a mobile phone (or about 50 m from a corresponding base station antenna). Therefore the restrictions being imposed on emission standards are with respect to continuous wave frequencies, but not with respect to a pulsed type, the latter being important in transmitting any intelligent information. Moreover real GSM signals are not constant in frequency and intensity. This distance of 20-30cm from the mobile phone corresponds to a distance of 20 to 30 m from a base station antenna. Panagopoulos et al (2010) showed that the bioactivity of GSM radiation in regard to short-term exposure is evident for radiation intensities down to 1μW/cm^2. This value of radiation intensity is encountered at about 1m distance from a cell phone or about 100 m distance from a corresponding base station antenna. This radiation intensity is 450 times and 900 times lower than the ICNIRP limits for 900 and 1800 MHz respectively (ICNIRP,1998). It has been estimated by Panagopoulos (2011) that people may be exposed to this level of radiation for long distances so, a factor of ten could be added as a safety factor, thereby bringing down the above figure to 0.1 μW/cm^2 , suggesting a limit for public exposure. These results support the findings that GSM radiation caused increased permeability of the blood –brain barrier in rat nerve cells and the strongest effect was produced by the SAR values which correspond to the weakest radiation intensity (Eberhardt et al.2008). The concept of window has earlier been described by Bawin et al (1978), Blackman et al (1980,1989). They have reported that the reproductive capacity decreases as the duration of exposure (1-21 minutes) increases(almost proportionally), for either of the two radiation types. Using statistical analysis they have confirmed that this variation is not because of the randomness of the subject, but because of the radiation exposure.

Several other authors have echoed a wide range of damaging effects on the male reproductive system and sperm parameters and cause significant changes in the sperm cell cycle (Derias et al 2006; Ji-Geng. 2007; Gutschi et al, 2011).

Non-genotoxic effects of Radiofrequency Radiation

Several studies reported no effect of RF fields on cell cycle kinetics (Vijayalaxmi et al 2001, Higashikubo et al 2001; Zeni et al, 2003; Miyakoshi et al, 2005; Lantow et al, 2006c). Alteration in cell proliferation was described only in a few reports (Pacini et al, 2002, Capri et al, 2004b).

Apoptosis is an important mechanism of protection against cancer. Several studies have reported RF field effects on human peripheral blood mononuclear cells (Capri et al, 2004a), lymphoblastoid cells (Marinelli et al, 2004), epidermis cancer cells (Caraglia et al 2005), and human Mono Mac 6 cells (Lantow et al, 2006c) and in Molts4 cells (Hook et al, 2004). No difference in apoptosis induction was detected between sham exposed and RF field exposed cells by Hook et al (2004). On the other hand, Marinelli et al (2004) have reported better survival rate of T lymphoblastoid leukaemia cells exposed to 900 MHz non-modulated RF fields and Carglia et al (2005) found apoptosis induction in human epidermoid cancer cells after exposure to 1.95 GHz fields. The European REFLEX study (Nikolova et al, 2005) reported no effects of RF fields on cell cycle, cell proliferation, cell differentiation, apoptosis induction, DNA synthesis and immune cell functionality. These authors described some findings after RF exposure on the transcript level of genes related to apoptosis and cell cycle control; however these responses were not associated with detectable changes of cell physiology. Analysis on whole genome cDNA arrays show alterations in gene expression after various RF exposure conditions using different cell types, but no consistent RF-signature such as stress response could be identified (Remondini et al, 2006).

Heat shock proteins act primarily as molecular chaperones to eliminate unfolded proteins, which can also appear from cellular stress. This stress response can be induced by many different external factors, including temperature, chemicals, oxidative stress, heavy metals, ionizing and non-ionizing radiation and ultrafine carbon black particles. Hsp70 has been shown to interfere with post mitochondrial events to prevent free radical mediated apoptosis (Gotoh et al 2001). An increased expression level of Hsp70 can thus offer protection against stress. Heat shock proteins are also involved in oncogenic processes (Jolly et al, 2000; Inoue et al, 1999; French et al, 2001).Some investigators have described increased heat shock protein level after RF exposure (Leszczynski et al, 2002; Kwee et al, 2001). However, these results are controversial, because there are negative findings also (Cotgreave 2005).

Nikolova et al (2005) described modulation in gene regulation after RF field's exposure at a SAR of 1.5 W/kg in p53-deficient embryonic stem cells. Proteomic analyses of human endothelial cell lines showed RF fields induced changes in this expression and phosphorylation state of numerous proteins including the hsp27.

Mitochondrial generation of ROS : DNA fragmentation and Effects

Free radical formation and their interaction with biological system is a matter of major concern for it has health implications. There is evidence of free radical generation after RF-microwave exposures (Phillips et al 2009; De lullis et al 2009;Kesari and Behari 2012,Kesari et al 2012).

Mitochondrial respiratory chain is the major site for the generation of superoxide radicals (O_2 and H_2O_2). It is possible that EMF may affect the mitochondrial membranes to produce large amount of radicals ROS under experimental conditions. EMF may disturb ROS metabolism by increasing the production of ROS or by decreasing the activity of antioxidant enzymes. From the data presented here it is obvious that such a change in testes that is highly dependent on oxygen to drive spermatogenesis and yet highly susceptible to the toxic effects of reactive oxygen metabolites, activity of anti-oxidant enzymes, and increases in ROS production. Reactive oxygen species (ROS) such as superoxide anions (O^-), hydroxyl radicals (OH^-) and hydrogen peroxide ($H_2 O_2$) may influence the structural integrity and function of sperm, such as motility, capacitation, and sperm-oocyte fusion (Griveau et al 1995). Spermatozoa are particularly vulnerable to oxidative stress because their plasma membrane is rich in polyunsaturated fatty acids (PUFAS) and membrane bound NADPH oxidase. Increased ROS production has been shown to correlate with reduced male fertility (Iwasaki and Gagnon 1992), to cause perioxidative damage to the sperm plasma membrane (Hughes et al 1996), and induce both DNA strand breakages and oxidative base damage in human sperm (Kodama et al 1997). A decrease in total antioxidant capacity of seminal plasma has been correlated with a reduction in sperm quality, such as concentration, motility and morphology (Smith et al 1996).

Since the most abundant molecule in biological cells is that of water (H_2O) microwave radiation can generate free radicals like OH^-, O^-_2, H, and H^-. These molecules are extremely reactive, having a tendency to react with different biomolecules including DNA, because of an unpaired electron that they comprise, which try to give up this extra charge and go into the paired mode. Also hydrogen peroxide (H_2O_2), a product of oxidative respiration in the mitochondria, which can be converted by electromagnetic radiation(EMR)into hydroxyl free radical via the Fenton reaction catalyzed by iron within the cells:

H_2O_2 + (EMR)-----→OH^-+OH^-

ROS generated by mobile phone exposure if not scavenged may lead to widespread lipid, protein, and DNA damage (Jajte et al 2002).

A summary of these results on Effects of Radiofrequency Microwave Radiation on Fertility and Reproduction is presented in Table 2.

The sequence of events leading toward infertility

A wide range of studies extending up to 50 GHz (Kesari and Behari 2009)) suggest that the DNA interaction with EMF is similar in nature across wide frequency ranges. DNA appears to possess the two structural characteristics of fractal antennas, electronic conduction and self- symmetry (Blank and Goodman 2011). These properties contribute to greater reactivity of DNA with EMF in the environment. The DNA damage could account for cancer promotion.

While damage to DNA has been confirmed in numerous scientific studies, it is argued that DNA repair is an on-going process and the damaged chromosomes can be reconstituted. However, this proposition is not without risk. There is no guarantee that these will replicate in the manner they were originally present. Pieces may be left out (deletions), joined in the backwards (inversions), swapped between different parts of the chromosomal (translocations)

Table 2: Overall effect of microwave radiation on reproduction and fertility

Organism used	**Mode of exposure**	**Parameters studied**	**Conclusion**	**Reference**
Fetus in the womb	laptop computers (LTCs)	induced currents in the body	power supply produces strong intracorporal electric current in the fetus and in the mother	Bellieni et al 2012
Sperm	Cell phone	Serum free testosterone (T), follicle stimulating hormone (FSH), luteinizing hormone (LH) and prolactin (PRL)	Higher T and lower LH levels No change in FSH and PRL values	Gutschi et al, 2011
Male Wistar rats	2.45 GHz	Creatine and caspase	Increase in caspase and creatine kinase ; decreases in testosterone and melatonin	Kesari et al, 2011
human spermatozoa	900-MHz	Acrosomal reaction, Morphometric parameters	affect sperm morphometry decrease in sperm	Falzone et al, 2011
Male Sprague Dawley rat	1.95 GHz 5 h/d for 5 weeks	SOD, CAT, GPx, histone kinase,Apoptosis	No testicular toxicity.	Imai et al. 2011
male mice	mobile phone base stations	sperm head abnormalities	knobbed hook, pin-head and banana-shaped sperm head	Otitoloju et al, 2010
Drosophila melanogaster	GSM 900MHz and DCS 1800MHz	Reproductive capacity	cumulative effects on living organisms.	Panagopoulos and Margaritis, 2010

Table 2 continued ..

Drosophila melanogaster	900 MHz	ovarian size	Significant reduction in size of ovary	Panagopoulos and Margaritis 2010
Male Wistar rat	900 MHz 2 h d for 45 day	Sperm count, apoptosis	Reduced sperm count and increased apoptosis	Kesari et al 2010
Male Wistar rat	50GHz	SOD, CAT, GPx, histone kinase,Apoptosis	Decreased SOD,GPX and Histone kinase, increased CAT and apoptosis	Kesari and Behari 2010
Male rabbit	800 MHz 8 h /d 12 weeks	Sperm count, weights of testis, epididymis, seminal vesicles, and prostate	Drop in sperm count	Salama et al 2010
Male and female mice (C57BL)	1966 MHz (UMTS)	Semen analysis and sperm function tests	No change	Sommer et al 2009
Rat	mobile phones	volumes of the ovaries and follicles	reduction in number of follicles	Gul et al, 2009
human spermatozoa	1.8 GHz	motility and vitality	mitochondrial reactive oxygen species generation	De Iuliis et al , 2009
Wistar albino male rats	900 MHz 2 h/day (7 days/week) for 10 months	Apoptosis of testes	No effect on caspase-3 levels	Dasdag et al. 2008

Table 2 continued…

Male Wistar rat	50-GHz microwave radiation 2 h a day for 45 days at a power level of 0.86 µW/cm^2	DNA strand break, Apoptosis	Increased apoptosis and DNA strand break	Kesari & Behari, 2008
Male Sprague-Dawley rats	cellular phone emissions	sperm motility, sperm cell morphology, total sperm cell number, and mRNA levels	abnormal clumping of sperm cells	Yan et al 2007
Male Sprague-Dawley rats	cellular phone emissions for 18 weeks	sperm motility, sperm cell morphology, total sperm cell number, and mRNA levels	sperm cell death and , abnormal clumping of sperm cells	Ji-Geng et al , 2007
Mice	1800 MHz	Serum testosterone	No detectable changes	Forgács et al.2006
Human semen	cell phone	Semen analyses	negative effects on the sperm motility	Fejes, et al 2005
Male NMRI mice	1800 MHz(100µW 2 h	Steroidogenic Leydig cells	No change	Forgács et al 2005
Drosophila melanogaster	900-MHz	Reproductive capacity	decrease cellular processes during gonad development	Panagopoulos et al 2004
Pregnant rats	915MHz microwaves	uteroplacental circulation, and in placental endocrine and immune functions	No effects on blood estradiol and progesterone,	Nakamura et al, 2000
Sprague-Dawley rats	cellular phones 20 min per day (7 days a week) for 1 month	malondialdehyde ,p53 immune reactivity, sperm count, morphology,	No significant alteration	Dasdag et al, 2003

or even attached to the wrong chromosome. The effect may also be frequency dependent. In most cases, the new arrangement can work for a while if most of the genes are still present and any metabolic deficiencies can often be made good by the surrounding cells. However, things may be different if it comes to meiosis. During meiosis, the chromosomes line up in pairs (one from each original parent) along their entire length so that corresponding parts are adjacent and can be exchanged. Malformed pairs are torn apart in the later stages of meiosis so that eggs or sperms have an incomplete or unbalanced set of genes, may not function properly and so reduce fertility and other physiological functioning. There is a possibility that this may lead to permanent genetic damage, which though may not be visible in the first generation but may be thereafter. A summary of these results on Effects of Radiofrequency Microwave Radiation on Fertility and Reproduction is presented in Table 3.

Table 3: Overview of effects of Microwave radiation on reproductive patterns

Parameter studied	**900 MHz**	**2.45GHz**	**10GHz**	**50GHz**
PKC	↓	-	-	-
SOD	↓	↓	↓	↓
CAT	↑	↑	↑	↑
GPx	↓	↓	↓	↓
H1K	↓	-	↓	↓
DNA damage	↑	↑	↑	-
ROS	↑	↑	↑	-
CK	↑	↑	↑	-
Testosterone*	↓	↓	↓	-
Caspase*	↑	↑	↑	-

↑ Indicates significant increase ↓ Indicate significant decrease

(PKC: Protein kinase C; ODC: Ornithine decarboxylase; SOD: Superoxide dismutase; CAT: Catalase; GPx: Glutathione peroxidase; H1K: Histone kinase, CK: creatine kinase, ROS: reactive oxygen species)

* Some studies have reported that there is no significant changes in reproductive system.

* Forgács et al 2005,2006 (1800 MHz)

* Dasdag et al. 2008 (900 MHz)

* Imai et al. 2011 (1.95 GHz)

* Sommer et al 2009 (1966 MHz, UMTS)

VI. PRUDENT AVOIDANCE AND GUIDANCE FOR SAFETY LIMITS

While it appears to have been convincingly established that electromagnetic fields have adverse biological effects on fertility and reproduction, the emphasis is on ‘use with caution’ rather than no use at all. Children in the age 12 years and younger are more prone to the damage because of their developing nervous system. Senior citizens and persons who are ill should also exercise caution and use wireless devices only in a most demanding situation. Mobile phones should thus be carried in close proximity of the body only in an OFF position (not ON and transmitting on standby). This is so because in an “standby” mode the phone emits signal intermittently - every few minutes they emit a periodic signal lasting a few seconds long - to maintain connection with the nearest base station antenna. These periodic signals are as powerful as the usual “talk signal” during a conversation. The user must make use of mobile phone speaker mode and keep the handset at least 40 cm away from their heads and other most sensitive organ like the head, heart and reproductive organs. Another method of protection (e.g. wired ear phones) are less effective, because of the existence of intensity window. The base station antennas should not be located within or near residential areas or near heavily populated areas. If antenna placement in the vicinity of residential zones is essential, they should be made to operate at substantially lowered power. Powerful wireless antennas should be placed on the hilltops and far from populated areas . The focus thus then shifts to prudent avoidance i.e. on to reduce the frequency and length of phone calls and keep away from these devices when not in use.

Bellieni et al (2012) have quoted that levels of exposure from “laptop” computers are higher than exposures that can be found in the proximity of high-voltage power lines and transformers or the domestic video screens .It has been observed that the magnetic field strength from power supplies is higher than that recommended by ICNIRP (1998) guidelines but that from LTC are within safe limits. It is thus suggested that use of LTC in an inclined position below the table level be avoided because it may cause increase in genital temperature ,besides causing back pain and fatigue. Moreover ‘laptop’ is a misnomer for its use in close proximity to the body is harmful.

Guidelines for Safety Limits

While considering the far field exposures, there are two sources: one is the microwave exposure from the base stations. While mobile phone exposure is localized, intermittent and is under voluntary control of the user, radiation from base towers is involuntary, whole-body and occurs 24 hours a day. While both the exposures may involve the same carrier frequency, the exposures are basically different in type and duration. On the whole it can be concluded that long term exposure near base stations can affect well-being of populations around them. Symptoms mostly associated with such exposures are headaches, tremor, restlessness and sleeping disorders.

The question of laying down the criteria for safe exposure is a problematic one, because the dose needs to be assessed not just as external field frequency (and spectrum), intensity, but also as cumulative exposure, as well as SAR, for whole body and specific anatomical sites. Accurate knowledge of RF exposure in a given scenario is needed for several parameters. The effect is not immediately visible but acts as silent killer. Any epidemiological studies for a long period (ten years or more) are difficult to carry under controllable situation, and few unexposed populations can serve as controls (non-exposed). Moreover the basic restrictions are expressed in quantities that are internal to the body and are not measured such as SAR. On the other hand, the reference levels are expressed (measured) in the free space situation, such as electric field. It is evident that SAR-concept alone is insufficient to define the safety guidelines for chronic exposure from mobile communications.

VI. CONCLUSIONS

Though causal evidence of one or more mechanism(s) are not yet fully refined, it is generally accepted that oxidative stress and free radical action may be responsible for the recorded genotoxic effects of EMFs which may lead to impairments in fertility and reproduction. Free radical action and/or hydrolytic enzymes like DNAase induced by exposure to EMFs may constitute the biochemical actions leading to adverse changes in hormones essential in males and female reproduction, DNA damage, which in turn causes damage to sperm motility, viability, and sperm morphology. Such exposures are now common in men who use and who wear wireless devices on their body, or use wireless-mode laptop computers. It may also account for damage to ovarian cells and female fertility, and miscarriage in women (ELF-EMF at 16 mG intermittent exposure).

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SECTION 19

Fetal and Neonatal Effects of EMF

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I. INTRODUCTION

The exposure of the developing fetus and of children to electromagnetic fields (EMF) including both radiofrequency radiation (RF) used in new wireless technologies, and to extremely low frequency or power frequency fields (ELF-EMF) has raised public health concerns because of the possible effects (cancer, neurological effects, developmental disability effects, etc) from the long-term exposure to low-intensity, environmental level fields in daily life. This chapter documents some studies on RF and ELF-EMF that report bioeffects and adverse health impacts to the fetus, and young child where exposure levels are still well within the current legal limits of many nations. Several studies report adverse health effects at levels below safety standards [Kheifets and Oksuzyan, 2008; Comba and Fazzo, 2009; World Health Organization. 2007]**;** the evidence to date suggests that special attention should be devoted to the protection of embryos, fetuses and newborns who can be exposed to many diverse frequencies and intensities of EMF throughout their lifetimes, where the health and wellness consequences on these subjects are still scarcely explored.

The studies of fetuses and newborns are an important subset of those made on older children. Infants' exposure to EMF has raised concern recently, and some countries have developed guidelines to limit it, by avoiding the presence of hospitals or schools within a certain range of kilometers around high EMF emission sources [http://www.emfs.info/Related+Issues/limits/]. Nevertheless, children and babies are chronically exposed to many sources of EMF, in particular at home, where they can spend much time playing with computers and other wireless-enabled devices, watching television or near electronic baby monitors that emit RF in their cribs (or sleeping areas). These exposures are relatively new in the last two decades, and may represent a potential new carcinogen and neurotoxin, that, with chronic and indiscriminate exposure, may have health consequences in the long term.

II. EMF AND RISK OF TUMORS

The evidentiary basis for evaluating an association between RF EMF exposure and brain cancer in children is much smaller than for adults [Wiedemann P, et al. 2009]. There is only one study available for mobile phone use. Elliott et al. [2010] found no association between risk of early childhood cancers (leukemia and non-Hodgkin's lymphoma, cancer of brain and central nervous system) and mothers' exposure to mobile phone base stations during pregnancy. Studies investigated brain cancer or leukemia with respect to EMF emitted from TV or radio transmitters

[Hocking et al. 1996; Dolk H, et al.1997; Cooper D, et al. 1997; Michelozzi P, et al. 2002; Park et al. 2004; McKenzie et al. 1998; Cooper et al. 2001; Maskarinec et al. 1994].

Few studies showed a significant increase of brain cancer in children with the use of cellular phones [Söderqvist et al. 2011; Merzenich et al. 2008], while some evidence exists for an association of RF EMF exposure to childhood leukemia. The argument for a causal influence of RF EMF exposure on leukemia in children is based on studies that found a statistically significant association between RF EMF exposure from radio or TV transmission towers and childhood leukemia. For instance, one case-control study [Ha, 2007.] found a significant increase for lymphocytic leukemia, but not for myelocytic leukemia in the highest exposure category.

Some authors suggested that genetic susceptibility to leukemia may amplify the adverse effects of magnetic field exposure, namely that the magnetic fields may have a causal role in the aetiology of leukemia among a genetically susceptible subgroup (i.e., children). For instance, Mejia-Arangure et al. [2007] observed a significant increase of childhood acute leukemia among Down syndrome subjects resident in dwellings with levels of magnetic flux density over 0.6 μT (OR= 3.7; 95% CI: 1.05-13.3). A recent paper [Kheifets and Oksuzyan, 2008] specifically addresses leukemia and it indicates as a priority the study of highly exposed children who live in apartments next to built-in transformers or electrical equipment rooms, emphasizing the investigation of joint effects of ELF environmental exposure and genetic co-factors.

III. EMF AND GENERAL HEALTH

Some studies address the question whether RF EMF exposure might cause general health disturbances in children [Milde-Busch et al. 2010; Heinrich et al. 2008; Divan et al. 2008; Söderqvist, 2008; Thomas, 2010; Vrijheid et al. 2010]. In a cross-sectional study Koivusilta et al. [2007] examined in a representative sample of 12–18-year-olds the association of mobile phone use with self- reported health status. Intensive use of communication technology, especially of mobile phones, was associated with health problems;. Van den Buick [2007] conducted a cohort study to assess the association between phone use by adolescents after lights out and levels of tiredness. Participants were adolescents with an average age of 14 in the youngest group and 17 in the oldest group. The authors found that those who used the mobile phone for calling and sending text messages after lights out were more likely to be very tired. Nevertheless, the results of these two studies were not proven to be due to EMF.

1V. EMF AND COGNITIVE FUNCTIONS

Original papers address the effect of RF EMF on cognitive function and CNS in children [Krause et al. 2006; Thomas et al. 2010; Abramson et al. 2009]. The age of the children investigated in these studies was in the range of 10–17 years. The argument supporting a causal influence of EMF exposure on cognitive function in children is based on the studies by several authors [Krause et al. 2006; Thomas et al. 2010; Abramson et al. 2009]. Lee et al [2001] administered three different tests that measure attention to 72 adolescents, who reported to either use a mobile phone or not. They found a statistically significant effect for one, the *Trail Making Test*. For the other two tests administered in the study, no statistically significant effects were found. The evidence for effects of RF EMF exposure on cognitive performance and CNS of children so far does not provide substantial hints for exposure-related changes. The very limited but provocative studies we do have suggest we cannot rule out that RF EMF exposure might influence cognitive and other CNS functions in children. If it is so, the consequences to public health can be enormous, if ignored.

V. FETUSES, NEWBORNS AND EMF

The early phases of human development have scarcely been studied with regard to their correlation with EMF. Nevertheless, the very young should receive more attention because of greater fragility and susceptibility of the developing embryo, fetus, and young child to environmental toxins of all kinds. Since fetuses and babies have a high number of stem cells and scarce immunity-mediated resources, any threat –in particular those due to physical and chemical agents – can have surprising and detrimental effects, since the environment influences even the DNA epigenetic expression [Davis and Lowell, 2008]. Czyz et al [2004] reported that GSM cell phone exposure affected gene expression levels in embryonic stem cells (p53-deficient); and significantly increased heat shock protein HSP 70 production. Belyaev et al [2010] reported that 915 MHz microwave exposure significantly affects human stem cells and may be important as a cancer risk. “The strongest microwave effects were always observed in stem cells. This result may suggest both significant misbalance in DSB repair, and severe stress response. Our findings that stem cells are the most sensitive to microwave exposure, and react to more frequencies than do differentiated cells may be important for cancer risk assessment and indicate that stem cells are the most relevant cellular model for validating safe mobile communication signals.”

In an animal study of mice, Aldad et al [2012] added support in a to the hypothesis that in-utero, whole-body exposure to RFR from cell phone radiation of the pregnant mother can result in hyperactivity, impaired memory and behavioral changes in the offspring.

Infante-Rivard and Deadman [2003] showed that maternal EMF exposure during pregnancy increased the risk of children 0-9 years of age developing leukemia (OR = 2.5, 95% CI = 1.2-3.0, for children of mothers in the highest 10% of exposure). Divan et al. [2008] reported that even prenatal exposure to cell-phone frequencies was associated with a significant increase in behavioral problems of emotion and hyperactivity around the age of school entry (OR = 1.80, 95% CI = 1.45-2.23). Although the results need replication, they point out an elevated susceptibility of the fetus and suggest a variety of adverse effects of cell-phone frequencies beyond just cancer. A recent study assessed that the exposure to EMF in pregnancy is linked to subsequent babies' asthma [Li et al. 2011].

Some researchers studied the possible effects of the exposure of fetuses to Magnetic Resonance Imaging (MRI) [Pediaditis et al. 2008]. Data seem to show that during abdominal MRI exposure limits of the mother "is not sufficient to protect the fetus if limits of the general populations are applied to it". In that case, fetal whole-body SAR exceeds limits by 7.4-fold. It is up to the physician and/or the ethics commission to decide upon justification for abdominal MRI of pregnant women if public safety limits are exceeded. The results indicate the need for specifically addressing fetal exposure to EMF and refining general recommendations by radiation protection bodies in line with the emerging science. Since the infant and young child are particularly vulnerable in general than adults, more care is needed to screen out unnecessary medical imaging of the pregnant woman and child and limit it to what is clearly medically necessary.

VI. LAPTOP COMPUTERS AND FETUSES

Bellieni et al [2012a] assessed EMF exposure levels of the 26-week fetus in the womb of a pregnant woman using a laptop computer in tight contact with pregnant women's belly. The word "laptop" means "a portable, usually battery-powered microcomputer small enough to rest on the user's lap," and this means that they are often used at close contact with the body in a very delicate area close to skin, bones, blood, genitals, and in the case of a pregnant woman, very close to her fetus. Since LTCs are often used in tight contact with the body even by pregnant women, fetal exposures to extremely low frequency (ELF-EMF) magnetic fields and induced electric currents within the fetus are generated by these units. These fields pass directly through the mother's tissues to the fetus. We measured the ELF-EMF emissions in five models of portable computers of different brands. Experiments were performed using a NARDA ELF 400 electromagnetic field measuring system (1 Hz to 400 kHz range) after determining the ambient background level was no higher than 0.01 μT. The point of highest emission was measured at the surface of the laptop. The voxel model used to calculate intracorporal electric current density distributions was a whole-body human database of average pregnant woman, jointly developed by the National Institute of Information and Communications Technology and Ciba University, which represents a pregnant woman at the 26th week of gestation. In this model, mother and fetus tissues are defined according to NICT (National Institute of Information and Communications Technology) pregnant female voxel phantom. Dielectric properties of mother tissues are calculated using the parametric model developed by C. Gabriel and colleagues that reproduces the tissue conductivities in a wide range of frequencies. In the five brands of LTC we examined, ELF-EMF levels for their dominant frequency ranges from 1.8 to 6 μT, whereas those produced from the power supply ranges from 0.7 to 29.5 µT.

Induced electric currents were estimated for both the pregnant woman and the fetus. Statistical values of the averaged current density were evaluated for body tissues including the body of the fetus, and the grey and white matter of the brain of the mother; the mother's cerebellum, the mother's cerebrospinal fluid and mother's muscle tissue. In each case, the larger exposure was generated by the power supply rather than the laptop operation. Levels of induced current substantially exceeded ICNIRP public safety limits, assuming close proximity of the laptop to the belly of the pregnant woman (for the fetus, between 182% and 263.7% of the ICNIRP standard); and for the woman (between 346.7% and 483.5% of the ICNIRP standard).

Simple measures to distance the laptop during use (placing it on a table or desk and not on the body of the user) will result in significant reduction of ELF-EMF exposure and induced electric current in both mother and fetus.

VII. NEWBORN (INFANT) INCUBATORS

Fetuses can also be born prematurely, and very often are protected in neonatal incubators for several weeks. Only a few studies of incubators (or isolettes) have assessed ELF-EMF magnetic field exposures to the newborn baby inside an incubator where the source is a motor that generates these emissions. The motors of neonatal incubators produce electromagnetic fields in their vicinity. Although premature babies are often exposed to incubator ELF-EMF for months, little research has been done into the effects of EMFs on newborns, and most has regarded newborn animals [Luchini and Parazzini, 1992; Watilliaux et al. 2011; Orendáčováet et al. 2011; Miyakoshi et al. 2012] so that the impact of this emission on the developing body's enhanced sensitivity to environmental insult is still largely unknown. In order to determine safe distances, ELF-EMF emissions must be measured and mapped, and these exposures need to be reduced to levels below that reported to cause adverse health effects in children (at or below 0.01 μT). To allow what is an essential medical intervention for the growing premature baby, or the sick infant who needs exceptional care following birth, at least two possible solutions to reduce ELF-EMF levels are:

- Designing incubators with the motor far from the baby (some incubators already have adopted this measure) and
- Using ELF-EMF absorbing panels to shield the baby's body from emissions (like Mu metal).

In Bellieni et al [2003], ELF-EMF levels are characterized in some common neonatal incubators. Levels of magnetic flux density at mattress level well over 10 milliGauss (mG) at mattress level: up to 88.4 mG in common incubators, and up to 357.0 mG in a transport incubator. These values are in line with those of two previous studies on ELF-EMFs in infant incubators[Lie and Kjaerheim, 2003; Babincova et al. 2000; Lie and Kjaerheim, 2003], and higher than the values recorded in two other reports [Aasen et al. 1996; Ramstad et al. 1998]. Another paper showed that nurses are also exposed to high EMF while working near incubators [Bellieni, 2002].

Bellieni et al [2008] reported that the exposure to high electromagnetic fields can interfere with the sympathetic nervous system in altering babies' heart rate variability. Heart rate variability (HRV) of 43 newborns in incubators was studied. HRV is an index of Autonomous Nervous System activity. The study group comprised 27 newborns whose HRV was studied throughout three 5-minute periods: 1) with incubator motor on, 2) with incubator off, and 3) with incubator on again, respectively. Mean HRV values obtained during each period were compared. The control group comprised 16 newborns but exposed to no source of ELF-EMF; they were exposed to changes in background noise similar to those provoked by the incubator motor (to reproduce the conditions of the first cohort). Mean total power and the high-frequency (HF) component of HRV increased significantly and the mean low-frequency (LF)/HF ratio decreased significantly when the incubator motor was turned off. Basal values were restored when incubators were turned on again. Changes in background noise did not provoke any significant change in HRV. We therefore concluded that ELF-EMFs produced by incubators influence newborns' HRV, showing an influence on their autonomous nervous system. More research is needed to assess possible long-term consequences, since premature newborns may be exposed to these high ELF-EMFs for months.

Even melatonin production – as was signaled in adults [Wilson et al. 1989] – was inhibited in the newborn by exposure to ELF-EMF [Bellieni et al. 2012b]. The study concerned 28 babies (study group), who had spent at least 48-hr in common incubators with the presence of significant ELF-EMF. Measurements of mean 6-hydroxy-melatonin-sulfate (6OHMS) urine excretion were recorded at the end of their stay in the incubators, and compared with their mean 6OHMS excretion after having been put in cribs, where EMF are below the detectable limit (<0.01 μT). Mean 6OHMS/cr values were respectively 5.34±4.6 and 7.68±5.1ng/mg (p=0.026) when babies were exposed to ELF-EMF in incubators, and after having been put in the crib. We have compared these changes with a control group of babies, who were not exposed to EMF either before the first sampling nor before the second. We therefore measured urine 6OHMS twice, with an interval of 48-hr, in a control group of 27 babies who were not exposed to EMF during both samples. In the control group, mean 6OHMS/cr values in the first and in the second sample were respectively 5.91±5.41 vs 6.17±3.94 ng/mg (p=0.679). The transitory increase in melatonin production soon after removing newborns from incubators demonstrates a possible influence of EMF on melatonin production in newborns. We should point out that the two groups were similar in all but their mean corrected age. It was greater in the control group (the time as measured from conception).

VIII. CONCLUSIONS

Some studies [Lowenthal et al. 2007; Infante-Rivard and Deadman, 2003] report that the fetus and young children are at greater risk than are adults from exposure to environmental toxins. This is consistent with a large body of information showing that the fetus and young child are more vulnerable than older persons are to chemicals [Makri A, et al. 2004] and ionizing radiation [Preston, 2004]. These considerations have led the US Environmental Protection Agency (EPA) to propose a 10-fold risk adjustment for the first 2 years of life exposure to carcinogens, and a 3-fold adjustment for years 3 to 5 [http://www.epa.gov/sab/pdf/sab_04003_resp.pdf].

This susceptibility may be why, according to some authors (60)[Carpenter and Sage, 2008], “the evidence for the relation between magnetic field exposure and leukemia in children is stronger than that for adults”.

The World Health Organization Agency International Agency for Research on Cancer (or IARC) classifies both ELF-EMF and RF EMF as Possible Human Carcinogens or Group 2B [http://microwavenews.com/news/backissues/j-a01issue.pdf]. These proposed US EPA adjustments do not deal with fetal risk, and the possibility of extending this protection to the fetus should be examined, because of fetus' rapid organ development. Classification of these related electromagnetic field exposures (ELF-EMF and RF EMF) as having the potential for serious potential health consequences for adults certainly justifies additional protections for the fetus, the newborn and young children who have greater sensitive to such exposures. Further, there is good evidence to suggest that many toxic exposures to the fetus and very young child have especially detrimental consequences depending on when they occur during critical phases of growth and development (time windows of critical development), where such exposures may lay the seeds of health harm that develops even decades later. See Appendix 1 for international statements of concern and delineation of priority research needs published by the WHO and US National Academy of Sciences, National Research Council.

Important bioeffects and some adverse health effects of chronic exposure to low-intensity (non-thermal) non-ionizing radiation have been reported on babies, and important open questions still remain.

Existing FCC and ICNIRP public safety limits seem to be not sufficiently protective of public health, in particular for the young (embryo, fetus, neonate, very young child).

The World Health Organization International Agency for Research on Cancer has classified both ELF-EMF and RF EMF (wireless radiofrequency) as Possible Human Carcinogens (Group 2B).

New, biologically-based public exposure standards are critically needed.

Common sense measures to limit both ELF-EMF and RF EMF in these populations is needed, especially with respect to avoidable exposures like incubators that can be modified; and where education of the pregnant mother with respect to laptop computers, mobile phones and other sources of ELF-EMF and RF EMF are easily instituted.

It is not in the public interest to wait: A precautionary approach may provide the frame for decision making where remediation actions have to be realized to prevent high exposures of children and pregnant woman.

APPENDIX 1

INTERNATIONAL STATEMENTS

World Health Organization Research Agenda for Radiofrequency Fields (2010) Children and EMF: Related Recommendations by World Health Groups

In 2010, the WHO produced a research agenda to address growing scientific questions and public concern about health effects of radiofrequency radiation, particularly with the explosive rise in exposures from new telecommunications technologies. It replaced a 2006 research agenda developed by the International EMF Project.

Priority: Epidemiology

***High -** Prospective cohort studies of children and adolescents with outcomes including behavioural and neurological disorders and cancer*

Rationale: As yet, little research has been conducted in children and adolescents and it is still an open question whether children are more susceptible to RF EMF since the brain continues to develop during childhood and adolescence. also, children are starting to use mobile phones at a younger age, given the existence of large-scale cohort studies of mothers and children with follow-up started during or before pregnancy, an RF sources component could be added at a reasonably low cost. Billing records for mobile phones are not valid for children, therefore the prospective collection of exposure data is needed. for neuropsychological studies, one challenge is to distinguish the "training" of motor and neuropsychological skills caused by the use of a mobile phone from the effects of the RF field. any future study should try to address this issue. in any case it should be of longitudinal design, thereby allowing the study of several outcomes and changes in technology and the use of mobile phones as well as other sources of RF EMF exposure, such as wireless laptops.

Priority: Human studies

***High** - further RF EMF provocation studies on children of different ages*

Rationale: current research has focused primarily on adolescents; very little is known about possible effects in younger children. longitudinal testing at different ages, for example by studying children already participating in current cohort studies, is recommended. This would allow consideration of the influence of potentially confounding factors such as lifestyle.

Priority: Animal studies

***High** - Effects of early-life and prenatal RF exposure on development and behaviour*

Rationale: There is still a paucity of information concerning the effects of prenatal and early life exposure to RF EMF on subsequent development and behaviour. Such studies are regarded as important because of the widespread use of mobile phones by children and the

> *increasing exposure to other RF sources such as wireless local area networks (Wlans) and the reported effects of RF EMF on the adult EEG. Further study is required which should include partial (head only) exposure to mobile phones at relatively high specific absorption rate (SAR) levels.*

National Research Council, National Academy of Sciences (2008)

The U.S. Food and Drug Administration (FDA) of the Department of Health and Human Services asked the National Academies to organize a workshop of national and international experts to identify research needs and gaps in knowledge of biological effects and adverse health outcomes of exposure to radiofrequency (RF) energy from wireless communications devices. To accomplish this task, the National Academies appointed a seven-member committee to plan the workshop (Committee on Identification of Research Needs Relating to Potential Biological or Adverse Health Effects of Wireless Communications Devices.). In their report, the Committee recommended these actions with respect to RF exposure for the developing fetus, and for young children:

- Characterization of exposure to juveniles, children, pregnant women, and fetuses from personal wireless devices and RF fields from base station antennas.

- Prospective epidemiologic cohort studies of children and pregnant women.

- Epidemiologic case-control studies and childhood cancers, including brain cancer.

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SECTION 20

Findings in Autism (ASD) Consistent with Electromagnetic Fields (EMF) and Radiofrequency Radiation (RFR)

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I. INTRODUCTION

The premise of this review is that although scant attention has been paid to possible links between electromagnetic fields and radiofrequency exposures (EMF/RFR) and Autism Spectrum Disorders (ASDs), such links probably exist. The rationale for this premise is that the physiological impacts of EMF/RFR and a host of increasingly well-documented pathophysiological phenomena in ASDs have remarkable similarities. Additional support may be found in the parallels between the rise in reported cases of ASDs and the remarkable increases in EMF/RFR exposures over the past few decades. Reviewing these similarities does not prove that these parallels imply causality – that kind of research has not been done. Moreover, the physiological processes affected by EMF/RFR are also impacted by other environmental factors. Yet EMF/RFR does not need to be a unique contributor to ASDs to add significantly to system overload (‘allostatic load’) and dysfunction. Even so these pathophysiological overlaps do suggest that the potential for an EMF/RFR-ASD connection should be taken seriously, and that their vulnerable biological features may make many with ASDs more likely to experience adverse EMF/RFR impacts. This is a sufficient basis to recommend that precautionary measures should be implemented and respected, that further research should be prioritized, and that policy level interventions based on existing and emerging data should be designed and pursued. Moreover, pursuing this link could help us understand ASDs better and find more ways to improve the lives of people with ASDs and of so many others.

A. How are Biology and Behavior Related?

Considering a potential link between ASDs and EMF/RFR (or indeed of any potential contributor to incidence or pathogenesis) requires taking account of the evolution that has been occurring in our understanding of the relationship between ASD’s behavioral and biological features. ASDs were first labeled as ‘autism’ in 1943 by Leo Kanner, a child psychiatrist who extracted several key behavioral features, related to communication and social interaction challenges and a tendency toward restricted interests and repetitive behaviors, characteristic of all 11 of the children in his first case series (Kanner 1943). Although in the seven decades since this condition was first constructed as a category there has been some modification of the way these behavioral features have been characterized, ASDs are still defined behaviorally, although sensory issues such as hypo- or hyper-reactivity have recently been included in the diagnostic criteria (Diagnostic and Statistical Manual of Mental Disorders or DSM-V) (American Psychiatric Association 2000, 2013, May).

1. Transduction is fundamental but poorly understood

Yet in considering how an environmental factor such as EMF/RFR could lead to autism and/or influence its severity or incidence, we need to think about how underlying biology is transduced into changes in nervous system electrical activity, and how these in turn generate the set of behaviors we have categorized as 'autism.' (Herbert 2005) This means not taking behaviors as given, or as purely determined by genetics, but exploring the full range of biology that generates these features and challenges.

2. More than brain

Although 'autism' has long been considered to be a psychiatric or neurological brain-based disorder (Rapin and Katzman 1998; Polleux and Lauder 2004), it has become undeniable that people diagnosed with ASDs often also have a multitude of biological features – including systemic pathophysiological disturbances (such as oxidative stress, mitochondrial dysfunction and metabolic and immune abnormalities) (Ming et al. 2012; Tsaluchidu et al. 2008; Pieczenik and Neustadt 2007; Gonzalez et al. 2011) as well as symptomatic medical comorbidities (such as gastrointestinal distress, recurrent infections, epilepsy, autonomic dysregulation and sleep disruption) (Nikolov et al. 2009; Kotagal and Broomall 2012; Kaartinen et al. 2012; Daluwatte et al. 2012; Tuchman and Cuccaro 2011; Canitano 2007; Malow 2004; Kang and Barnes 2013; Jyonouchi et al. 2011) – in addition to the core defining behaviors – and many of these occur commonly (Kohane et al. 2012). The problem has been that no one such biological feature has turned out to be present in every single person carrying an ASD diagnosis – and they are not specific to ASDs, either. Moreover there has been much variability in many of the features of autism – not only between individuals but in many cases within individuals at different points in time or under different circumstances. Because of this variability, the relevance of many of these biological features has been dismissed as secondary and not intrinsically related to the 'autism.' Instead, many have considered the behavioral features as fundamental not only to how autism manifests and is definedbut also to the core intrinsic nature of ASDs, even though the biological basis of these behaviors has by no means been established.

3. Heterogeneity: More genetic and environmental than physiological

It is not as if this variability is unique to the 'environmental side.' At the present time over 800 genes have been associated with ASDs, and over 100 different rare genetic syndromes are frequently accompanied by ASD, with no clear specific unifying mechanism uniting this remarkable heterogeneity (Trikalinos et al. 2006; Ring et al. 2008; Pelphrey et al. 2011; Mandell 2011; Hall et al. 2012; Bill and Geschwind 2009). Similarly a large number of potential environmental contributors are under investigation ranging from toxicants and Vitamin D deficiency or failure to take prenatal vitamins to air pollution and stress or infection in pregnancy (Whitehouse et al. 2012; Kocovska et al. 2012; Schmidt et al. 2011; Landrigan 2010; Roberts et al. 2007; Shelton, Hertz-Picciotto, and Pessah 2012; Becerra et al. 2012; Volk et al. 2011). Yet at the physiological level a smaller set of disturbances are showing up as common across substantial numbers of people with ASDs – and in fact not uniquely to ASDs but also in myriad other chronic conditions whose prevalence also appears to be increasing (Bilbo Jones, and Parker 2012; Knox 2010). Prominent among these are immune disturbances including inflammation, mitochondrial dysfunction, and oxidative stress, as well as toxic body burden. Vulnerability to all of these can be increased mildly or substantially by a variety of often common genetic mutations, but may remain latent without the overlay of environmental triggers. Conversely, with substantial enough environmental input, genetic vulnerability may not be necessary.

4. Mechanism is more than correlation

Just HOW biological features might be related to the behavioral features that have up until now defined ASDs has not been clarified; until recently the main research effort regarding pathophysiology in ASDs has been to establish the presence of these phenomena in the first place. Even so, some correlations between biological and behavioral features have been identified – e.g. a higher level of immune abnormalities correlates with more aberrant behaviors (Wei et al. 2012; Careaga and Ashwood 2012; Jyonouchi et al. 2011; Ashwood et al. 2011; Heuer et al. 2008; Zerrate et al. 2007; Curran et al. 2007). Still, such correlations in themselves do not explain the *mechanisms* by which the *transduction of pathophysiology into behavior* might actually occur. In order to do that, an important component would be to study the relationship between systemic pathophysiology and nervous system electrophysiology.

5. EMF/RFR research may help us understand how ASDs 'work'

Assessing the potential contribution of EMF/RFR to ASDs puts this question of the nature of the pathophysiology-behavior transduction into an interesting and provocative light since the brain is simultaneously a tissue-based physical organ that can be compromised by cellular pathophysiology as well as altered developmental processes, and an information processing system that operates through networks of synchronized electrical oscillations (brain waves) – and EMF/RFR impacts may occur directly at both of these levels. To date the emphasis in ASD research has largely been on 'structure-function' relationships that have been anatomy-centered. This research has generated correlations between brain structures and behaviors, and has found some genetic correlates as well, but it has made assumptions that these phenomena are rooted in genetics and genetically perturbed molecular structures and substances. This leads to targeting the molecular level with pharmaceuticals, but not to the broader agenda of understanding environmental or physiological contributions or dynamic features of brain and behavior. Thus, exploring how EMF/RFR impacts ASDs may help to force the question of how these pathophysiological and electrophysiological/information processing levels actually interact, and how anatomy may in many ways be a product rather than a cause of physiology.

B. Time Courses of Mechanisms

For the most part, researchers have looked for causes of autism in mechanisms that occur early and create permanent change or damage. This approach is logical if one assumes that genetic influences are overwhelmingly predominant, and 'autism' is a fixed lifelong trait. However evidence is emerging that ASDs may in many respects be more state-like and variable than trait-like and fixed.

1. Plasticity

One of the remarkable shifts in conceptual thinking about ASDs is an appreciation of its brain plasticity (Helt et al. 2008). Growing numbers of reports of improvement and loss of diagnosis, reversal of neurological symptoms in a growing number of mouse models of genetic syndromes that in humans prominently feature autism (Cobb, Guy and Bird 2010; Ehninger et al. 2008; Goebel-Goody et al. 2012; Henderson et al. 2012; Kaphzan et al. 2012; Liu, huang, and Smith 2012; Mehta, Gandal, and Siegel 2011; Paylor et al. 2008; Rotschafer et al. 2012; Sato et al. 2012; Suvrathan et al. 2010), short-term pharmaceutically induced improvement in brain connectivity (Narayanan et al. 2010), and transient reversal or abeyance of symptomatology under various circumstances (including fever, fluid-only diet, and certain antibiotic treatments (Sandler et al. 2000; Curran et al. 2007)) – all of these throw into question the long-standing assumption that we are simply dealing with a 'broken brain.' Indeed, how could a 'broken brain' produce markedly improved function with such a short turnaround time? (Herbert 2009) Such a time frame cannot possibly be accounted for by remodeling of the brain's anatomical substrate. 'Brain waves' and their synchronization, on the other hand, could easily vary over short time periods. Looking into physiological and environmental modulators not only of brain development but also of everyday brain function becomes increasingly imperative.

In addition, documentation of average to superior intelligence in most people with autism (Edelson 2006; Dawson et al. 2007), as well as of domains of perceptual superiority (Soulieres, Zeffiro, et al. 2011; Soulieres, Dawson et al. 2011; Samson et al. 2011; Soulieres et al. 2010; Soulieres et al. 2009; Mottron et al. 2006; Mottron 2004; Bertone et al. 2005; Perreault et al. 2011), call into question the long-standing assumption that ASDs are intrinsically or for the most part associated with cognitive deficits – another strike against the outdated 'deficit' or 'broken brain' model.

2. Mechanisms that operate actively throughout the lifecourse

One particularly valuable lesson about ASDs that can be learned from looking at how EMF/RFR affects underlying biology is that these impacts are by no means confined to early development. We already have clinical reports of 'intermittent autism' – for example, some children with mitochondrial disease who have ups and downs of their bioenergetics status 'have autism' on their bad days but don't display autistic features on their good days (Korson 2007). These children with their vulnerable, barely compensated mitochondria seem to be teetering right at the brink of the interface of metabolic and electrophysiological dysfunction, tipping back and forth on this knife edge. It makes one wonder what everyday exposures – allergens, infection, pesticide on the school playground, even perchance EMF/RFR – might contribute to the bad days (with their loss of electrophysiological optimization, probably on account of insufficient energy to drive fully integrated brain function), and conversely how many choices exist in everyday life that could tilt things in the direction of more good days (by helping to stabilize more optimal nervous system performance) (Herbert and Weintraub 2012).

The short time course needed for biologically effective EMF/RFR 'doses' to lead to observable impacts reflects that these exposures can affect cells without obstruction (unlike many chemical agents), and create impacts within minutes. This type of mechanism may also give us fresh and important ways of understanding the short-term variability – the good days and the bad days – that are so common in ASD even in those who do not have a formal diagnosis of mitochondrial disease.

3. Pathophysiology and allostatic load

Based on these considerations, the strategy to be pursued in this examination of a potential EMF/RFR - ASD link is to review the many parallels between underlying biology, or pathophysiology, in ASDs and the impacts of EMF/RFR on living organisms. EMF/RFR exposures have demonstrated impacts at just about every level at which biology and physiology have been shown to be disrupted in ASDs. EMF/RFR has been shown to potentiate the impact of various toxicants when both exposures occur together (Juutilainen, Kumlin, and Naarala 2006); this may be additive or more than additive. This suggests that EMF/RFR may synergize with other contributors and make things worse. With many different environmental factors piling on to a much smaller number of environmentally vulnerable physiological mechanisms (Herbert 2010), one must consider that the model of 'allostatic load' – the sum total of stressors and burdens – may be central to understanding how the many risk factors interact to create autism – and to create a spectrum of levels of severity across so many of ASD's associated features. A cascade of exposures interacting with vulnerabilities can potentially lead to a tipping point for an individual, such as the phenomenon of autistic regression experienced by a substantial subset of people with ASDs. When exposures increase at the population level, we are likely to see trends of increase in the number of people passing that tipping point and getting diagnosed. EMF/RFR exposures have increased several thousand-fold or more in the past two decades from wireless technology innovations that have unplanned side effects from pulsed RFR, a newly classified human carcinogen (Baan et al, 2011). Nearly six billion people globally own wireless phones, for example. Many hundreds of thousands more are exposed to wireless whole-body transmissions from wireless antenna facilities (Sage and Carpenter, BioInitiative 2012 Report, Section 24). For this as well as for physiological reasons allostatic loading as a viable concept for the study of ASDs should reasonably address EMF/RFR as one of the collection of exposures of relevance to the overall stress load, since it is now a chronic and unremitting exposure in daily life at environmentally relevant levels shown to cause bioeffects from preconception and pregnancy through infancy, childhood and the whole lifecourse.

In an article entitled "Unrelenting Stress is Toxic,: The New Scientist (28 July 2012) describes stress in an eloquent way:

> "*Unrelenting stress is toxic because it can turn the body's defense system against itself. Neuroendocrinologist Bruce McEwen at Rockefeller University in New York says the stress response that evolved to protect us from harm can be hijacked and actually cause harm when the stress level never abates. In a normal situation, the introduction of stress causes the body to deliver a boost of energy – by sending a surge of glucose to the muscles – and to increase heart rate, blood pressure and breathing to get oxygen to the muscles in hurry. At the same time, blood vessels constrict and clotting factors increase – ready to slow bleeding in case you are wounded. These responses are a part of a fight-or-flight survival kit, and once the stress has passed, these should subside. But for people under unrelenting stress, this response never quite switches off – leaving sugar levels unregulated, high blood pressure, increate risk of blood clots, depressed sex drive and an immune system buckling under the strain. Prolonged exposure to stress hormones can have other effects as well, including affecting the brain by altering the structure of the neurons and their connections, which in turn can influence behaviour and hormonal processes."*

This passage refers to effects on the hypothalamo-pituitary-adrenal axis (Aldad, 2012), but as will be discussed in the Part II, equally important is cellular stress from stress proteins (heat shock protein HSP) and from oxidative stress generated at very low-intensity EMF and RFR levels as detailed in the BioInitiative 2012 Update, Section 7 by Martin Blank, PhD; Blank, 2012). Both are significant kinds of stress that can add body-burdens via allostatic loading.

II. PARALLELS IN PATHOPHYSIOLOGY

This section will review parallels in pathophysiology between ASDs and impacts of EMF/RFR. It will begin with a review of mechanisms of direct impact at the level of molecules, cells, tissues and genes. It will then move on to consider how these levels of damage lead to degradation of the integrity of functional systems including mitochondrial bioenergetics, melatonin, immune function and nervous system physiology. The review of parallels will conclude with a discussion of electromagnetic signaling and synchronized oscillation from membranes to nervous system, treating 'aberrant' neural systems and somatic function and behaviors as consequences or 'outputs' of disturbed underlying physiology to which EMF/RFR is a plausible contributor.

A. Damage: Means and Domains

ASDs have been conceptualized as 'neurodevelopmental' which has focused attention on how genes and environment could alter brain development. This leads to the unstated presumption that virtually everything important about the brain in ASDs has to do with differences in the way it was formed. In genetics this has led to a hunt for neurodevelopmental genes. There is no question that environmental impacts can alter brain development, and impact brain function across the lifespan. This chapter begins the work to systematically rectify the omission of EMF/RFR as one environmental contributor in ASDs.

However the influence of the environment on neurodevelopmental conditions such as ASDs does not stop there. Evidence is accumulating showing that increased expression of genes associated with physiological dysregulation, as well as single-nucleotide polymorphisms (SNPs) associated with these issues, may be if anything more prominent than alterations of 'neurodevelopmental' genes (Lintas, Sacco, and Persico 2012). In a study of gene expression in ASDs, Down syndrome and Rett syndrome, these authors state, *"Our results surprisingly converge upon immune, and not neurodevelopmental genes, as the most consistently shared abnormality in genome-wide expression patterns. A dysregulated immune response, accompanied by enhanced oxidative stress and abnormal mitochondrial metabolism seemingly represents the common molecular underpinning of these neurodevelopmental disorders."* Others have also found pathophysiology-related genes as figuring most prominently in alterations of gene expression in ASD (Kong et al. 2012; Jung, Kohane, and Wall 2011; Voineagu et al. 2011; Waly et al. 2012). SNPs associated with methylation abnormalities, impaired glutathione synthesis and mitochondrial dysfunction also have been identified as significant risk factors.

Genetics may create risk, but the actual nervous system and health consequences probably come from dysfunction at the physiological level. Evidence for pathophysiological dysfunction in ASDs increasingly abounds. In particular, a growing body of literature documents immune aberrations, low total and reduced glutathione levels, lower activity of the anti-oxidative stress system and mitochondrial dysfunction. These phenomena may be both genetically and environmentally modulated. As will be discussed further below, they are certainly pertinent to the neurodevelopment of the brain, which has been by far the dominant focus autism research, but it does not stop there as they can significantly modulate brain function in real time, as well as shape the function of the entire organism, including the autonomic system, the cardiovascular, endocrine, immune, gastrointestinal and reproductive systems and more.

1. Cellular Stress

Oxidative Stress

Autism (ASD) research indicates that oxidative stress may be a common attribute amongst many individuals with autism. In the past decade the literature on this has moved from a trickle to a flood. Studies document reduced antioxidant capacity, increased indicators of oxidative stress and free radical damage, alterations in nutritional status consistent with oxidative stress, altered lipid profiles, and pertinent changes not only in blood but also in brain tissue. Associations of ASDs with environmental exposures such as air pollution and pesticides are indirectly supportive as well, since such exposures are linked in other literature to oxidative stress (Kanthasamy et al. 2012; Roberts et al. 2010; Knox 2010; Rose, Melnyk, Trusty, et al. 2012; Rose, Melnyk, Pavliv, et al. 2012; Ghanizadeh et al. 2012; Frustaci et al. 2012; Rossignol and Frye 2011; Adams et al. 2011, 2011; Mostafa et al. 2010; Zecavati and Spence 2009; Yao et al. 2006; Naviaux 2012; Chauhan and Chauhan 2006; Chauhan, Chauhan, and Brown 2009).

Reactive oxygen species are produced as a normal consequence of mitochondrial oxidative metabolism as well as other reactions, but when their number exceeds the cell's antioxidant capacity a situation of oxidative stress develops. It is certainly the case that oxidative stress can be a consequence of exposures to chemical toxicants, or of the interactive impacts of toxicants, nutritional insufficiencies and genetic vulnerabilities. This set of risk factors has received considerable attention for the potential roles each component and various possible combinations could play in causing or exacerbating autism.

Less often mentioned in the ASD pathophysiology literature is that it is also well established that EMF/RFR exposures can be associated with oxidative damage.

Published scientific papers that demonstrate the depth of EMF and RFR evidence reporting oxidative damage in human and animal models are profiled in Section 6 (Genotoxicity) of this BioInitiative 2012 Report and in the BioInitiative Report (2007), both by Henry Lai, PhD (Lai, 2012; Lai, 2007). These cellular effects can occur at low-intensity, legal levels of exposure that are now 'common environmental levels' for pregnant women, the fetus, the infant, the very young child, and the growing child as well as for adults. Electromagnetic fields (EMF) can enhance free radical activity in cells (Lai and Singh 2004; De Iuliis et al. 2009) particularly via the Fenton reaction, and prolonging the effect causes a larger increase, indicating a cumulative effect. The Fenton reaction is a catalytic process of iron to convert hydrogen peroxides, a product of oxidative respiration in the mitochondria, into hydroxyl free radical, which is a very potent and toxic free radical (Lai, in the BioInitiative Report 2007; Lai, 2007). Free radicals damage and kill organelles and cells by damaging macromolecules, such as DNA, protein and membrane components.

Further indications of a link to oxidative stress are findings that EMF and RFR at very low intensities can modulate glutamate, glutathione and GABA, and affect mitochondrial metabolism. Alterations in all these substances and processes have been documented in ASDs (Bristot Silvestrin et al. 2012; Brown et al. 2012; Choudhury, Lahiri, and Rajamma 2012; Essa et al. 2012; Oberman 2012; Yang and Pan 2012; Chauhan, Audhya, and Chauhan 2012; Frustaci et al. 2012; Main et al. 2012; Pecorelli et al. 2012; Rose, Melnyk, Pavliv, et al. 2012; Rose, Melnyk, Trusty et al. 2012; Waly et al. 2012; Banerjee et al. 2012; Coghlan et al. 2012; Enticott et al. 2012; Kang and Barnes 2013; Mendez et al. 2012; Piton et al. 2012; Anitha, Nakamura, Thanseem, Matsuzaki, et al. 2012; Anitha, Naamura, Thanseem, Yamada, et al. 2012; Gargus 2008; Giulivi et al. 2010; Hadjixenofontos et al. 2013; Napolioni et al. 2011; Rossignol and Frye 2011). Campisi et al (2010) report that increased glutamate levels from 900 MHz cell phone frequency radiation on primary rat neocortical astroglial cell cultures induced a significant increase in ROS levels and DNA fragmentation after only 20 min with pulsed RFR at non-thermal levels (Campisi et al. 2010).

Fragopoulou et al (2012) conducted proteomics analysis of proteins involved in brain regulation in mice as a consequence of prolonged exposure to EMF (Fragopoulou et al. 2012). They identified altered expression of 143 proteins, ranging from as low as 0.003 fold downregulation up to 114 fold overexpression with affected proteins including neural function-related proteins including Glial Fibrillary Acidic Protein (GFAP), alpha-synuclein, Glia Maturation Factor beta (GMF), apolipoprotein E (apoE)), heat shock proteins, and cytoskeletal proteins (i.e., neurofilaments and tropomodulin), as well as proteins of brain metabolism such as aspartate aminotransferase and glutamate dehydrogenase. The authors pointed out that oxidative stress was consistent with some of these changes.

Aberrations in glutathione metabolism and deficiencies in reserves of reduced glutathione are increasingly associated with ASDs, both systemically and in the brain. The parallel with EMF/RFR impacts here is strong, since glutathione reduction associated with EMF/RFR is reported in at least twenty three relevant research studies in both human and animal studies since 1998, including the following citations (Shapiro et al. 2012; Ozgur, Guler, and Seyhan et al. 2010; Ozguner et al. 2005; Moustafa et al. 2001; Kesari, Kumar, and Behari 2011; Jelodar, Akbari, and Nazifi 2012; Hoyto et al. 2008; Guney et al. 2007; Esmekaya et al. 2011; Atasoy et al. 2012)Al-Demegh, 2012; Kumar, 2010; Meral, 2007; Oktem et al. 2005; Ozguner et al. 2006). It is increasingly appreciated that glutathione is a final common pathway, a critical piece of environmentally vulnerable physiology, as glutathione reserves are compromised by an enormous number of environmental stressors, so that the cumulative impact upon glutathione may be far greater than could be predicted by the magnitude of any specific exposure (Lee, Jacobs, and Porta 2009), which supports an allostatic loading model.

Also of note are studies showing that the effects of EMF/RFR can be reduced by supplementation with antioxidants and radical scavengers. As an example, Vitamins E and C reduced adverse impacts on rat endometrium from 900MHz EMR exposure (Guney et al. 2007). Gingko biloba has also prevented mobile phone-induced increases in malondialdehyde and nitric oxide levels in brain tissue as well as decreases in brain superoxide dismutase and glutathione peroxidase activities and increases in brain xanthin oxidase and adenosine deaminase activities, and treated rats were spared the histopathological cell injury found in the untreated rats (Ilhan et al. 2004). Substantial further literature on antioxidants and radical scavengers is reviewed in Section 15 in Belyaev's contribution to the Bioinitiative 2012 Report (Belyaev 2012).

Stress protein (heat shock protein) responses

Another well-documented effect of exposure to low- intensity ELF and RFR is the creation of stress proteins (heat shock proteins) that signal a cell is being placed under physiological stress) (Weisbrot et al. 2003; Velizarov, Raskmark, and Kwee 1999; Leszczynski et al. 2004; Leszczynski et al. 2002; de Pomerai et al. 2000; Daniells et al. 1998; Blank and Goodman 2004). Heat shock proteins are in a family of inducible proteins that are initiated when any increased need for protection from stray electrons occurs (Padmini 2010; Bottoni, Giardina, and Scatena 2009). The HSP response is generally associated with heat shock, exposure to toxic chemicals and heavy metals, and other environmental insults. HSP is a signal of cells in distress. Plants, animals and bacteria all produce stress proteins to survive environmental stressors like high temperatures, lack of oxygen, heavy metal poisoning, and oxidative stress. It should also be noted that the generation of HSP stress proteins can have constructive medical applications, such as protection from reperfusion of the heart following ischemic injury (George et al. 2008). Another concomitant impact of cellular stress can be protein misfolding, which has been documented in association with exposure to EMF/RFR. (Bohr and Bohr 2000; Mancinelli et al. 2004)

Although a number of papers have demonstrated increases in HSPs in people with ASDs (El-Ansary and Al-Ayadhi 2012; Evers, Cunningham-Rundles, and Hollander 2002; El-Ansary, Ben Bacha, and Kotb 2012; Walker, Segal, and Aschner 2006; Vojdani et al. 2004), it has been investigated far less often than oxidative stress. Part of the research needed to study possible influences of EMF/RFR on ASDs would be to study this more carefully.

2. Membranes and channels

Cell membranes and lipid peroxidation

Cell and organelle membranes play roles in partitioning cells from the extracellular milieu as well as in sustaining boundaries and regulating flow of materials between cellular compartments needing different metabolic parameters for their activities. They also play critical roles in maintaining electrical differences and the flow of electricity.

Adey (2002) summarized studies that report cell membranes as the site of initial field transductive coupling.

> *"Collective evidence points to cell membrane receptors as the probable site of first tissue interactions with both ELF and microwave fields for many neurotransmitters (Mironova et al. 1994), hormones (Liburdy 1995; Ishido, Nitta, and Kabuto 2001), growth- regulating enzyme expression (Byus, Pieper, and Adey 1987; Chen et al. 2000; Litovitz et al. 1993) (Penafiel et al. 1997), and cancer-promoting chemicals (Cain, Thomas, and Adey 1993; Mevissen, Haussler, and Loscher 1999). In none of these studies does tissue heating appear involved causally in the responses. Physicists and engineers have continued to offer microthermal, rather than athermal, models for these phenomena (Barnes 1996; Astumian, Weaver, and Adair 1995), with views that exclude consideration of cooperative organization and coherent charge states, but it is difficult to reconcile experimental evidence for factors such as modulation frequency-dependence and required duration of an amplitude-modulated signal to elicit a response (coherence time) (Litovitz et al. 1993) with models based on the equilibrium dynamics of tissue heating."* (Adey 2002)

Membranes are well-known targets of oxidative stress. Membrane damage is a major route through which free radical damage proliferates through the cellular system. Lipid peroxidation of membranes most often affects polyunsaturated fatty acids such as EPA and DHA which are the most abundant and vulnerable lipids in the brain where the damage they sustain can have serious impacts – DHA is 40% of brain tissue. Lipid peroxidation of membranes has been identified as an effect of EMF/RFR in multiple studies (Desai, Kesari, and Agarwal 2009; Phelan et al. 1992). A variety of other mechanisms for membrane alteration related to EMF/RFR have been intimated in the literature. Physicochemical properties of membranes such as phase transition of phosphatidylcholine can be shifted by nonthermal effects of microwave radiation (Beneduci et al. 2012). Membrane potential and currents may also be impacted by pulsed radiofrequency fields (Linz et al. 1999). This has been observed graphically in altered cellular movement in Paramecium caudatum, with these cells becoming broader, with a broader-appearing cytopharynx, with their pulse vesicles having difficult in expelling their content outside the cell, and with less efficient movement of cilia (Cammaerts et al. (2011) which the authors suggested might be due to targeting of the cellular membrane. The impacts on this unicellular organism may help us imagine what the impact of EMF/RFR might be on cells with some structural similarities, such as columnar epithelial cells and ciliated cells in mucosal surfaces in the respiratory system, digestive tract, uterus and fallopian tubes and central spinal cord.

Indications of lipid peroxidation of membranes has been documented in ASDs, including malonaldehyde and isoprostanes, as well as alteration of membrane phospholipids and prostaglandins (Pecorelli et al. 2012; El-Ansary et al. 2010; El-Ansary, Ben Bacha, and Kotb 2012; Zhang, Sun, et al. 2012; Yao et al. 2006; Al-Gadani et al. 2009; Chauhan and Chauhan 2006; Ming, Stein, et al. 2005; Zoroglu et al. 2004) In one study the iosoprostane levels showed a biomodal distribution with the majority of ASD subjects showing moderate increase but a smaller group showing dramatic increases (Ming, Stein, et al. 2005). Thromboxane, reflecting platelet activation, was also elevated in one study (Yao et al. 2006). Given that this phenomenon has been identified in many people with ASDs, it is plausible that such individuals will likely be more vulnerable to having such cellular injuries caused, worsened or both by EMF/RFR exposures.

Calcium channels

Of particular prominence in the EMF/RFR physiological impact literature is the impact on calcium channels and signaling. Calcium signaling is ubiquitous in biological systems ranging from single-celled organisms to the most sophisticated functioning of our nervous and immune systems. This signaling takes place through a myriad of mechanisms within and between cells. The exquisite tuning of organisms is influenced by the precision of functioning of these systems, with even subtle disturbances having the potential to ramify in a nonlinear fashion through a system causing larger-scale disturbances elsewhere. EMF/RFR exposures have been shown to create disturbances in calcium signaling through a variety of mechanisms, including membrane leakage (Nesin et al. 2012), alteration of calcium-binding proteins and GFAP reactivity (Maskey et al. 2012; Maskey et al. 2010), and altered ultrastructural distribution of calcium and calcium-activated ATPases after exposure (Kittel et al. 1996). Adey (2002) provided an overview of key studies on calcium efflux and the importance of calcium in cell signalling. *"Early studies described calcium efflux from brain tissue in response to ELF exposures (Bawin and Adey 1976; Blackman et al. 1985), and to ELF-modulated RF fields (Bawin and Adey 1976) (Blackman 1979) (Blackman et al. 1985; Dutta, Ghosh, and Blackman 1989). Calcium efflux from isolated brain subcellular particles (synaptosomes) with dimensions under 1.0 µm also exhibit an ELF modulation frequency-dependence in calcium efflux, responding to 16 Hz sinusoidal modulation, but not to 50 Hz modulation, nor to an unmodulated RF carrier (Lin-Liu and Adey 1982). In the same and different cell culture lines, the growth regulating and stress responsive enzyme ornithine decarboxylase (ODC) responds to ELF fields (Byus et al. 1988; Litovitz et al. 1993) and to ELF-modulated RF fields (Byus, Pieper, and Adey 1987) (Litovitz et al. 1993) (Penafiel et al. 1997) ."* (Adey 1994)

Dutta et al (1992) reported:

> *"Radio-frequency electromagnetic radiation (RFR) at 915 and 147 MHz, when sinusoidally amplitude modulated (AM) at 16 Hz, has been shown to enhance release of calcium ions from neuroblastoma cells in culture. The dose-response relation is unusual, consisting of two power-density "windows" in which enhanced efflux occurs, separated by power-density regions in which no effect is observed. To explore the physiological importance of these findings, we have examined the impact of RFR exposure on a membrane-bound enzyme, acetylcholinesterase (AChE), which is intimately involved with the acetylcholine (ACh) neurotransmitter system. Neuroblastoma cells (NG108), exposed for 30 min to 147-MHz radiation, AM at 16 Hz, demonstrated enhanced AChE activity, as assayed by a procedure using 14C-labeled ACh. Enhanced activity was observed within a time window between 7.0 and 7.5 h after the cells were plated and only when the exposure occurred at power densities identified in a previous report as being effective for altering the release of calcium ions. Thus RFR affects both calcium-ion release and AChE activity in nervous system-derived cells in culture in a common dose-dependent manner."* (Dutta et al. 1992)

The prominence of these calcium signaling impacts of EMF/RFR are striking when considered in relation to ASD pathophysiology, where such alterations have been proposed as of central importance. Calcium channels play an important role in regulating neuronal excitability, whose disturbance during development has been thought by many to be potentially contributory to the development of ASDs, as well as to the often associated vulnerability to seizures. Gene alterations have been identified associated with a number of voltage-gated calcium channels in ASDs (Smith, 2012; Krey and Dolmetsch 2007; Pasca et al. 2011; Gargus 2009; Lu et al. 2012). However, based on an examination of patient laboratory and phenotype data it has been argued that aberrant calcium signaling could be downstream: Palmieri and Persico (2010) suggest that *"an* *abnormal neuroimmune response as a relevant player in elevating intracellular Ca2+ levels, deranging neurodevelopment, driving oxidative stress, and ultimately affecting synaptic function and neural connectivity especially in long-range neuronal pathways physiologically responsible for integrated information processing."* (Palmieri and Persico 2010) Peng and Jou (2010) have in turn shown how increased intracellular calcium can cause oxidative stress, and a vicious circle: *"...mitochondrial ROS [reactive oxygen species]rise can modulate Ca2+ dynamics and augment Ca2+ surge. The reciprocal interactions between Ca2+ induced ROS increase and ROS modulated Ca2+ upsurge may cause a feedforward, self-amplified loop creating cellular damage far beyond direct Ca2+ induced damage."* (Peng and Jou 2010*)*

Environmental as well as genetic routes to calcium signaling dysfunction have been identified (Pessah and Lein 2008) including chemicals such as the polyaromatic hydrocarbons. PCB-95 in particular modulates the calcium-dependent signaling pathway responsible for activity-dependent dendritic growth (Wayman, 2012; Wayman, 2012). In fact, once a genetic mutation has been associated with altering a critical signaling pathway and conferring risk for autism, chemicals or other environmental agents can be identified that target the same pathways and also confer ASD risk. Stamou et al. (2012) have reviewed this strategy of identifying multiple mechanisms converging on common signaling pathways regarding Ca(2+)-dependent mechanisms as well as extracellular signal-regulated kinases (ERK)/phosphatidylinositol-3-kinases (PI3K) and neuroligin-neurexin-SHANK (Stamou et al. 2012). From this point of view, there may be no particular reason to privilege genetic mutations in their contribution to a disturbance of calcium signaling, since whether this function becomes derailed due to a genetic mutation, from a chemical toxin or from EMF/RFR perturbation of calcium signaling, the functional effect is comparable. Moreover if a person is subject to multiple triggers all of which have calcium signaling impacts, the gene-environment interactions may lead to impacts that could be less, the same as or more than any one contributor alone might create.

3. Junctions and barriers

The damage discussed so far has been at the molecular and subcellular level. However impacts from this level reverberate up to larger scales in the system. Where membranes create boundaries between cells and subcellular compartments, barriers do this at a larger scale. Cells become capable of forming barriers between each other through tight junctions which block substances and cells from 'slipping through the cracks,' so to speak, between the cells. Conversely, gap junctions are subcellular structures providing openings that allow physical passage of materials between cells otherwise separated by membranes.

It appears that such connections between cells can also be altered by electromagnetic fields and radiofrequency exposures, at least under certain circumstances. High frequency magnetic fields have been observed to be associated with a sharp decrease in intercellular gap junction-like structures, in spite of increased gene expression for pertinent proteins (Cervellati, 2009). Changes in tight junctions have been observed upon exposure to microwave and x-ray irradiation {Palfia, 2001).

A number of papers in the ASD research field document problems pertinent to junctions. Connexin abnormalities have been documented in neuropathological studies (Fatemi et al. 2008). and MacFabe and colleagues identified lipid alterations associated with oxidative stress, membrane fluidity and the modulation of gap junction coupling (Thomas et al. 2012). Decrease in platelet endothelial cell adhesion molecule-1 were reduced and this reduction correlated with repetitive behavior and abnormal brain growth; achesion molecules modulate permeability and signaling at the blood-brain barrier as well as leukocyte infiltration into the central nervous system (Onore et al. 2012).

EMF and RFR might also compromise biologically important barrier structures that separate blood flow from organs like the brain (Salford et al, BioInitiative Report 2012, Section 10) (Salford, 2012). This raises important questions regarding whether other ‘barriers’ that keep blood flow separate from the gut (gut-blood barrier), or the placenta (blood-placenta barrier) or the eye (ocular-blood barrier) may also be rendered pathologically leaky, and allow albumin, toxins, pro-inflammatory cytokines and infectious agents to cross this barrier into the intestines (invoking immune responses) and impacting the developing fetus (Somosy, 1993). While there are a fair number of negative studies, there are also many studies showing and association between EMF/RFR and pathological leakage of the blood-brain barrier (BBB), as well as evidence in animal studies of damage to brain cells and damage to or death of neurons. Such leakage has been shown to be potentiated by physiological factors such as diabetes and insulin (Gulturk et al 2010) and has also potentiated viral lethality in a dose-dependent fashion (Lange et al, 1991). Many of the positive findings were associated with non-thermal exposures comparable to normal cell phone radiation exposure (Salford, 1994; Salford, 2003; Salford, 2007; Salford, 1992; Eberhardt, 2008; Nittby, 2009; Nittby, 2008). There are scattered reports of increased permeability across other membranes and barriers, such as the blood-testicle barrier in mice (Wang, 2008; Wang et al., 2010 and the rat liver canalicular membrane (Lange, 1993). A 1992 study by Kues et al. reported that *“studies in our laboratory have established that pulsed microwaves at 2.45 GHz and 10 mW/cm2 are associated with production of corneal endothelial lesions and with disruption of the blood-aqueous barrier in the non-human primate eye.”* (Kues et al. 1992) A recent study showing impact of high-frequency electromagnetic fields on trophoblastic connexins (Cervellati et al. 2009) may indicate the vulnerability of the placenta and placental barrier function to electromagnetic fields. A thorough review and methodological discussion of literature regarding EMF/RFR impacts on the BBB is provided by Salford in Section 10 of the BioIniative 2012 Report (Salford, 2012).

According to a review by Zlokovic, *"BBB breakdown, due to disruption of the tight junctions, altered transport of molecules between blood and brain and brain and blood, aberrant angiogenesis, vessel regression, brain hypoperfusion, and inflammatory responses, may initiate and/or contribute to a "vicious circle" of the disease process, resulting in progressive synaptic and neuronal dysfunction and loss in disorders such as Alzheimer's disease, Parkinson's disease, amyotrophic lateral sclerosis, multiple sclerosis, and others."* (Zlokovic 2008). The integrity of the BBB can be compromised by oxidative stress which can lead to increased permeability (Parathath, Parathath, and Tsirka 2006). The resultant extravasation of albumin into brain parenchyma can be excitotoxic and neurotoxic (Hassel, Iversen, and Fonnum 1994; Eimerl and Schramm 1991).

The evidence suggesting possible existence of barrier function compromise in people with ASDs is largely indirect. The existence of brain neuroinflammation in ASDs has been documented in a growing number of studies (Boso et al. 2006; El-Ansary and Al-Ayadhi 2012; Young et al. 2011), and this is known to be associated with BBB permeability (Erickson, Dohi, and Banks 2012; Janigro 2012; Takeshita and Ransohoff 2012). In a review of clinical MRI findings in ASDs 19/59 showed white matter signal abnormalities (Boddaert et al. 2009), which in other settings have been associated with cerebral hypoperfusion, though not necessarily in the same locations as the hyperintensities (Vardi et al. 2011; Brickman, 2009). Blood flow abnormalities, predominantly hypoperfusion, documented in a few dozen PET and SPECT studies, could also be caused by and/or associated with physiological phenomena associated with vascular permeability as will be revisited below. Increased intestinal permeability has been documented (although its absence has also been documented) (de Magistris et al. 2010; Lucarelli et al. 1995; D'Eufemia et al. 1996; Horvath and Perman 2002; White 2003; Robertson et al. 2008; Souza et al. 2012) and discussed in the context of food exposures, particularly gluten (Silva et al. 2012; Sapone et al. 2011; Visser et al. 2009; Simpson et al. 2009; Fasano 2009; Lammers et al. 2008; De Angelis et al. 2006). The reactivity to large numbers of different foods clinically observed in many children with autism has been framed by some as a manifestation of indiscriminate exposure of the immune system and the brain to food proteins on account of intestinal permeability as well as BBB permeability (Theoharides and Doyle 2008). This reactivity could in turn feed in to aberrant immune responsivity which in turn could further amplify barrier vulnerability (Fasano, 2009).

A number of studies have made an association between an increased risk of having a child with autism and maternal infection during pregnancy. This phenomenon looks like it is a result of the maternal immune system response rather than being due to an impact deriving from a specific infectious agent; but the potential for an accompanying compromise of the placental barrier is also conceivable in this setting. Under these circumstances the fetal risk of exposure to maternal blood toxins, cytokines and stress proteins in-utero could potentially be increased if placenta barrier (BPB) function were impaired. The integrity, or compromise thereto, of the maternal-fetal interface via the placenta is an important modulator of brain development (Hsiao and Patterson 2012).

4. Genetic alterations and reproductive impacts

Because of the high heritability of autism that was calculated from the concordance rates of monozygotic (identical) vs. dizygotic (fraternal) twins found in by a series of small twin studies performed some decades ago, the overwhelming emphasis in recent decades in autism research has been on genetics, and on finding linkages between genes, brain and behavior. As mentioned earlier, this point of view also promotes more of a structural/anatomical orientation than a bioelectric/physiological orientation. Along with this emphasis it has seemed obvious to people just looking at the stubborn persistence of symptoms in affected individuals that ASDs are inborn, lifelong brain defects. From this vantage point there would be no reason to think about the transduction of pathophysiology – whether acquired or genetic or some combination – to brain and hence behavior (or, more broadly, neurocognitive function). Thus the research agenda of looking for gene-brain-behavior correlations has seemed both self-evident and sufficient.

In recent years the genetic premises of this seemingly obvious framing of autism as overwhelmingly genetic have been undermined at several levels. (The undermining of the brain premises will be discussed beyond what was covered in Part I in later sections.) First the number of reported cases is increasing, making it more difficult to maintain that ASDs are purely genetic because these increases can only be partly explained away by greater awareness or other data artifacts (King and Bearman 2009; Hertz-Picciotto and Delwiche 2009). Second, the complexity of the ways we understand how genes might relate to autism has grown, from an expectation a decade ago that a small number of genes (even less than a dozen) would explain everything to an identification of close to a thousand genes associated with autism, as well as 'de novo' mutations present in ASD children but not their parents and even 'boutique' mutations not shared beyond an individual family. Out of over a hundred genetic syndromes in which autism commonly occurs, it is unclear what the pertinent genetic mutations and rearrangements have in common to account for the shared association with ASDs (Anney et al. 2010; Betancur 2011). Moreover, a recent twin study that was much larger than any of the prior such studies identified a modest genetic role but a substantial environmental role (Hallmayer et al. 2011). Also of interest, a Swedish study of identical twins and schizophrenia grouped into monochorionic (shared placenta) and dichorionic (each had its own placenta) showed 60% concordance for schizophrenia diagnosis for monochorionic twins but only 10.7%

concordance for dichorionic twins (Davis, Phelps, and Bracha 1995); though this work has not yet been replicated in ASD twins, in principle it opens the door to non-genetic interpretations of any concordance figures that have generally been assumed to be indicators of heritable genetics. The authors of this study interpreted their findings as consistent with data on viral infection as a contributor to schizophrenia risk (a possibility also entertained in ASDs (Patterson 2012; Teixeira and Barichello 2012; Atladottir et al. 2012, 2012; Hornig et al. 1999), but one could also consider the possibility of differences in the dichorionic cases in the integrity of the placental barrier.

All of this calls into question the idea that genetics can be presumed to be the 'cause' of autism simply based upon heritability calculations, and upgrades the importance of looking not only at the environment and environmentally vulnerable physiology, but also at acquired mutations. There is certainly progress being made through genetic research to the identification of networks of genes and mechanisms on which genes converge (Voineagu et al. 2011), but environmental mechanisms converge on these mechanisms too (Stamou et al. 2012), and the mechanisms are what drive the impacts.

Genotoxicity

One route through which environmental impacts may influence an organism's status is by changing genes through mutation – that is, by genotoxicity. This has been proposed as a mechanism for the generation of 'de novo' mutations (found in children but not their parents) being found in ASDs (Kinney et al. 2010) and increasingly in other settings as well, making mutations something that needs to be accounted for rather than simply assuming tey are associated with normal, stable variation. Reviews and published scientific papers on genotoxicity and EMF report that both ELF-EMF and RFR exposures can be considered genotoxic – i.e., damaging to DNA – under certain conditions of exposure, including under conditions of intermittent and/or chronic ELF and RFR exposure that are of low-intensity and below current world safety standards (Ruediger 2009; Ivancsits et al. 2005; Diem et al. 2005; Blank and Goodman 2011; Phillips, Singh, and Lai 2009; REFLEX 31 May 2004; Sage and Carpenter 2009; Lai and Singh 2004). Types of genetic damage reported have included DNA fragmentation and single- and double-strand DNA breaks, micronucleation and chromosome aberrations, all of which indicate genetic instability. Genotoxic impacts of EMF/RFR are further reviewed in the BioInitiative Working Group 2007 contribution by Lai as well as in Section 6 of the present Bioinitiative Report {Lai, 2007; Lai, 2012).

The European research program REFLEX (Risk Evaluation of Potential Environmental Hazards From Low-Energy Electromagnetic Field Exposure Using Sensitive in vitro Methods – a 5FP EU project) documented many changes in normal biological functioning in tests on DNA at exposure levels below existing public safety standards(REFLEX 31 May 2004). Some of the key findings included:

- Gene mutations, cell proliferation and apoptosis which are caused by or result in altered gene and protein expression profiles. The convergence of these events is required for the development of all chronic diseases.
- Genotoxic effects and a modified expression of numerous genes and proteins after EMF exposure could be demonstrated with great certainty.
- Genotoxic effects produced by RF-EMF in fibroblasts, HL- 60 cells, granulosa cells of rats and neural progenitor cells derived from mouse embryonic stem cells.
- Response of cells to RF exposure between SAR levels of 0.3 and 2 W/Kg with a significant increase in single- and double-strand DNA breaks and in micronuclei frequency.
- A clear demonstration of increase in intracellular generation of free radicals in HL-60 cells accompanying RF-EMF exposure.
- The observation that the induced DNA damage was not based on thermal effects, which raises concerns about the thermal-based environmental safety limits for ELF-EMF exposure.

These impacts could be contributors to a role for genetics in ASDs that does not derive from only inheritance but also from environmental and epigenetic influences. Moreover, in the light of the great heterogeneity of genetic findings in ASD alongside the documented impacts of EMF/RFR upon many other levels of pathophysiology than simply genetics, it becomes worth reflecting whether genetics might not be the primary problem but instead, in many cases at least, just one of many levels of collateral damage from environmental impacts. Whatever genetic variants a person carries may bias their system toward specific vulnerability, or may contribute more generically by increasing entropy and molecular disorder; in either capacity they may aggravate the situation but may not be part of the main cause.

Contributors to Genotoxicity

Oxidative stress and free radical damage to DNA

Oxidative stress and excessive free radical production are very well known to be potentially genotoxic. They can be a consequence of myriad environmental factors, including but by no means limited to EMF/RFR. The DNA damage that can result could very well be one cause of ‘de novo’ mutations. Although there is not a consensus at this time about the rates or causes of *de novo* mutations in ASDs, and using present methods of detection are only found in a small percentage of individuals with ASDs, given the potential contribution of environmentally triggered oxidative stress and free radical damage that we know is present in at least large numbers of people with ASDs, a serious investigation of the potential contribution of EMF and RFR to de novo mutations in ASD seems warranted, given the large increase in exposure to these phenomena accompanying the massively increased non-ionizing radiation exposures in daily life due to electrification and the global saturation of RFR from wireless technologies (BioInitiative 2012 Report, Section 24, Public Health Implications, Sage and Carpenter, 2012).

Challenge to DNA repair mechanisms

Reduced DNA repair may contribute to increased risk of cancers, but it may also contribute to a variety of other diseases and disturbances of growth and development. When the rate of damage to DNA exceeds the rate at which DNA can be repaired, there is the possibility of retaining mutations and initiating pathology. Failure to trigger DNA damage repair mechanisms, or incomplete or failed repair, may be a consequence of a variety of commonplace stressors, including EMF/RFR exposure. A decrease in DNA repair efficiency has been reported to result from exposure to low-intensity RFR in human stem cells, and other cells. Mobile phone frequency GSM exposure at the frequency of 915 MHz consistently inhibited DNA repair foci in lymphocytes (Markova et al. 2005; Belyaev et al. 2005; Belyaev, Markova, and Malmgren 2009). Belyaev, Markova and colleagues (2005) and Markova et al. (2009) reported that very low-intensity microwave radiation from mobile phones inhibits DNA repair processes in human stem cells. A significant reduction in 53BP1 ((tumor suppressor p53 binding protein 1) foci was found in cells exposed to microwave radiofrequency radiation within one hour of exposure. Fibroblast cells were impacted in this fashion but adapted over time, whereas stem cells were similarly affected (inhibited 53BP1 foci) but did not adapt to microwave radiation during chronic exposure (Markova et al. 2005; Belyaev et al. 2005). Additional challenges to DNA repair mechanisms include not only toxicants and other damaging inputs but also nutritional insufficiencies of substances important to the proper functioning of DNA repair mechanisms, including Vitamin D, essential fatty acids, and minerals such as selenium and molybdenum (Christophersen and Haug 2011). The high possibility that various such contributors may combine supports an ‘allostatic load’ model of environmental injury and genotoxicity. Also note the overlap between nutritional risk factors for oxidative stress and for impaired DNA repair mechanisms. This supports a vicious circle model where the more oxidative damage to the genome, the less the cells will be prepared to deal with it successfully. It can also work the other way around – nutrients can attenuate the degree of damage; instances of this will be discussed in the Melatonin section below.

Chromatin condensation

Chromatin condensation is another hallmark of damage from EMF and RFR. Orderly chromatin condensation is a normal part of cell division, but it can also be provoked pathologically. The work of Markova, Belyaev and others has repeatedly shown that RFR exposure can cause chromatin condensation. Belyaev (1997) reported that super-low intensity RFR resulted in changes in genes, and chromatin condensation of DNA at intensities comparable to exposures from cell towers (typically at RFR levels of 0.1 to 1.0 uW/cm2) (Belyaev, Alipov, and Harms-Ringdahl 1997). Significant microwave-induced changes in chromatin conformation were observed when rat thymocytes were analyzed in-between 30-60 min after exposure to MW (Belyaev and Kravchenko 1994). This effect nearly disappeared if the cells were incubated more than 80 min between exposure and analysis.

In recent studies, human lymphocytes from peripheral blood of healthy and hypersensitive to EMF persons were exposed to non-thermal microwave radiation NT MW) from the GSM mobile phones (Belyaev et al. 2005; Markova et al. 2005). NT MW induced changes in chromatin conformation similar to those induced by heat shock, which remained up to 24 h after exposure. The same group has reported that contrary to human fibroblast cells, which were able to adapt during chronic exposure to GSM/UMTS low intensity RFR exposure, human stem cells did not adapt (Belyaev, Markova, and Malmgren 2009).

Researchers have recently identified large numbers of “spontaneous genetic glitches,” or de novo mutations, more likely to be transmitted by fathers than by mothers to their children (Neale et al. 2012; O'Roak et al. 2012; Sanders et al. 2012). These glitches are widely distributed across the genome, with their location rather than their size conferring risk. The Eichler team at the University of Washington found that 39% of the 126 most severe or disruptive mutations map to a network associated with chromatin remodeling that has already been ranked as significant amongst autism candidate genes (O'Roak et al. 2012). Whether the prominence of chromatin-related gene mutations can be related in any meaningful way to the impacts of EMF/RFR on chromatin condensation is not possible to say at this point in time and this apparent parallel between ASDs and EMF/RFR may be a pure coincidence, though an intriguing one worth looking into further, including regarding how these mutations and the chromatin-remodeling impacts of EMF/RFR exposure may interact.

Gonadal and germline impacts

De novo mutations have been shown to be more of a problem related to paternal age (O'Roak et al. 2012; Paul, Nagano, and Robaire 2011; Iossifov et al. 2012; Cantor et al. 2007; Alter et al. 2011), and this may be related to the impact of environmental factors such as EMF/RFR on the stem cell genome, particularly in sperm which have no DNA repair capacity. Vulnerability of testes and ova, and of sperm and egg cells, relates to the tissue milieu in which damage to the germline can take place, as well as on the greater vulnerability of stem cells. Several international laboratories have replicated studies showing adverse effects on sperm quality, motility and pathology in men who use and particularly those who wear a cell phone, PDA or pager on their belt or in a pocket (Agarwal et al. 2008; Agarwal et al. 2009; Wdowiak, Wdowiak, and Wiktor 2007; De Iuliis et al. 2009; Fejes et al. 2005; Aitken et al. 2005) Kumar, 2012). Other studies conclude that usage of cell phones, exposure to cell phone radiation, or storage of a mobile phone close to the testes of human males affect sperm counts, motility, viability and structure (Aitken et al. 2004; Agarwal et al. 2007; Erogul et al. 2006). Animal studies have demonstrated oxidative and DNA damage, pathological changes in the testes of animals, decreased sperm mobility and viability, and other measures of deleterious damage to the male germ line (Dasdag et al. 1999; Yan et al. 2007; Otitoloju et al. 2010; Salama et al. 2009) Behari et al. 2006; Kumar et al. 2012). Of note, altered fatty acids consistent with oxidative stress have been found in sperm cells in male infertility (Zalata et al. 1998; Zalata, Hafez, and Comhaire 1995).

There are fewer animal studies that have studied effects of cell phone radiation on female fertility parameters. Panagopoulous et al. 2012 report decreased ovarian development and size of ovaries, and premature cell death of ovarian follicles and nurse cells in *Drosophila melanogaster* (Panagopoulos 2012). Gul et al (2009) report rats exposed to stand-by level RFR (phones on but not transmitting calls) caused decrease in the number of ovarian follicles in pups born to these exposed dams (Gul, Celebi, and Ugras 2009). Magras and Xenos (1997) reported irreversible infertility in mice after five (5) generations of exposure to RFR at cell phone tower exposure levels of less than one microwatt per centimeter squared (µW/cm2) (Magras and Xenos 1997).

Implications of genotoxicity

The issue of genotoxicity puts the contribution of genetic variation into a different light – as something that needs to be accounted for, not necessarily assumed as the starting point. In this regard it has been speculated that the apparent higher rates of autism in Silicon Valley, discussed in the past as related to 'geek genes' (Silberman 2001), might be conditioned by higher levels of exposure to EMF/RFR. The relationship between the greater vulnerability of male sperm than of female eggs to adverse effects of EMF/RFR exposure and the marked (4:1) predominance of paternal origin of de novo point mutations (4:1 bias), also deserves further careful attention (O'Roak et al. 2012).

5. Implications of Damage

We have reviewed parallels between ASD and EMF/RFR in molecular, cellular and tissue damage, including cellular stress (oxidative stress, the heat shock response and protein misfolding), injury of membranes, aberrant calcium signaling, and compromise of junctions and barriers. The genotoxicity of EMF/RFR was reviewed in relation to issues of environmental contributions to autism and of the phenomenon of de novo mutations. The compromise of the tissue substrate appears to have many commonalities in ASDs and in EMF/RFR exposures. Also notable was the possibility of attenuating some of the damage through increasing antioxidant status.

These commonalities come to mind in considering the implications of a recent study documenting arrest of symptomatology in a mouse model of Rett syndrome through a bone marrow transplant of wild-type microglia (Derecki et al. 2012; Derecki, Cronk, and Kipnis 2012). The introduction of these competent microglia cells did not directly target the neuronal defect associated with the MECP2 gene mutation; instead the benefits of the transplant were diminished through inhibition of phagocytosis. Phagocytosis involves removing debris. This suggests that while research has focused on how specific molecular defects, particularly in the synapse, may contribute to Rett pathophysiology, there may also be an important contribution from cellular debris, misfolded proteins and other disordered cellular structure and function. Such disorder could be accumulating in cells under the conditions of pathophysiological disarray reviewed above. This has potentially broad implications for other genetic disorders, as well as for conditions like ASDs which are for the most part idiopathic. Based on this study as well as on the levels of damage just reviewed, problems in cells that are pertinent to ASDs most likely go beyond any specific defect introduced by a mutation. Additionally it is conceivable that many of the mutations may be not part of normal background variation but instead collateral damage from the same environmental factors that are also driving the damage to the pathophysiology. It is also encouraging that at least some of the damage and dysfunction was reversible by a generic cellular mechanism (phagocytosis), and this could have broad significance for idiopathic ASDs as well, along with other conditions involving related pathophysiological challenges.

B. Degradation of System Integrity

In the setting of molecular, cellular and tissue damage, one would predict that the organization and efficiency of a variety of organelles, organs and systems would also be degraded. EMF/RFR exposures yield a stressful situation of chronically interrupted homeostasis. Here we will review disturbances from EMF/RFR in systems (including include oxidative and bioenergetics metabolism, immune function and electrophysiological oscillations) that include molecular and cellular components subject to the kinds of damage discussed in the previous section. We will review disturbances that have been associated with EMF/RFR, and consider the parallel disturbances that have been documented in ASDs.

1. Mitochondrial dysfunction

Mitochondria are broadly vulnerable, in part because the integrity of their membranes is vital to their optimal functioning – including channels and electrical gradients, and their membranes can be damaged by free radicals which can be generated in myriad ways. Moreover, just about every step in their metabolic pathway can be targeted by environmental agents, including toxicants and drugs, as well as mutations (Wallace and Starkov 2000). This supports an allostatic load model for conditions in which mitochondrial dysfunction is an issue, which includes ASDs as well as myriad other chronic conditions.

Mitochondria are commonly discussed in terms of the biochemical pathways and cascades of events by which they metabolize glucose and generate energy. But in parallel with this level of function there also appears to be a dimension of electromagnetic radiation that is part of the activity of these organelles. For example, electromagnetic radiation can be propagated through the mitochondrial reticulum, which along with the mitochondria has a higher refractive index than the surrounding cell and can serve to propagate electromagnetic radiation within the network (Thar and Kuhl 2004). It is also the case that *"The physiological domain is characterized by small-amplitude oscillations in mitochondrial membrane potential (delta psi(m)) showing correlated behavior over a wide range of frequencies…. Under metabolic stress, when the balance between ROS [reactive oxygen species, or free radicals] generation and ROS scavenging [as by antioxidants] is perturbed, the mitochondrial network throughout the cell locks to one main low-frequency, high-amplitude oscillatory mode. This behavior has major pathological implications because the energy dissipation and cellular redox changes that occur during delta psi(m) depolarization result in suppression of electrical excitability and Ca2+ handling…"* (Aon, Cortassa, and O'Rourke 2008). These electromagnetic aspects of mitochondrial physiology and pathophysiology could very well be impacted by EMF/RFR.

There are also a variety of types of mitochondrial damage that have been documented in at least some of the studies that have examined the impacts of EMF/RFR upon mitochondria. These include reduced or absent mitochondrial cristae (Khaki et al. 2006; Lahijani, Tehrani, and Sabouri 2009; Esmekaya et al. 2011), mitochondrial DNA damage (Xu et al. 2010), swelling and crystallization (Lahijani, Tehrani, and Sabouri 2009), alterations and decreases in various lipids suggesting an increase in their use in cellular energetics (Chernysheva 1987), damage to mitochondrial DNA (Xu et al. 2010), and altered mobility and lipid peroxidation after exposures (Wang et al. 2002). Also noted has been enhancement of brain mitochondrial function in Alzheimer's transgenic mice and normal mice (Dragicevic et al. 2011). The existent of positive as well as negative effects gives an indication of the high context dependence of exposure impacts, including physical factors such as frequency, duration, and tissue characteristics; these are intensively reviewed in Belyaev's contribution to BioInitiative 2012 in Section 15 (Belyaev 2012).

The idea that mitochondrial dysfunction might be common in ASDs met with a fair bit of consternation, and many professionals have preferred to limit their consideration to mitochondrial disorders with proven genetic mutations. However the concept of mitochondrial dysfunction is better established in other areas of medicine, with thousands of papers and hundreds of reviews carrying "mitochondrial dysfunction" in their titles. By now there is a large amount of evidence for biochemical and other abnormalities in a large portion of children with autism that are consistent with mitochondrial dysfunction (Giulivi et al. 2010; Palmieri et al. 2010; Pastural et al. 2009). Recently published postmortem brain tissue studies that have added a new dimension of evidence for mitochondrial abnormalities in ASDs will be reviewed in the section on alteration of brain cells below.

Some have called the mitochondrial issues most commonly seen in ASDs 'secondary mitochondrial dysfunction' (Zecavati and Spence 2009; Rossignol and Frye 2011) to indicate that it results from environment insults and/or other pathophysiological dysfunction rather than directly from genetics (Hadjixenofontos et al. 2012); the already discussed potential for EMF/RFR to damage channels, membranes and mitochondria themselves could contribute in a number of ways to degrading mitochondrial function without a basis in genetic mutation, as could toxicant exposures and immune challenges. In a meta-analysis of studies of children with ASD and mitochondrial disorder, the spectrum of severity varied, and 79% of the cases were identified by laboratory not associated with genetic abnormalities (Rossignol and Frye 2011). "*Substantial percentages of autistic patients display peripheral markers of mitochondrial energy metabolism dysfunction, such as (a) elevated lactate, pyruvate, and alanine levels in blood, urine and/or cerebrospinal fluid, (b) serum carnitine deficiency, and/or (c) enhanced oxidative stress……In some patients, these abnormalities have been successfully explained by the presence of specific mutations or rearrangements in their mitochondrial or nuclear DNA. However, in the majority of cases, abnormal energy metabolism cannot be immediately linked to specific genetic or genomic defects."* (Palmieri and Persico 2010)

2. Melatonin dysregulation

Melatonin, mitochondria, glutathione, oxidative stress

Melatonin is well-known for its role in regulation of circadian rhythms, but it also plays important metabolic and regulatory roles in relation to cellular protection, mitochondrial malfunction and glutathione synthesis. (Leon et al. 2005; Luchetti et al. 2010; Limon-Pacheco and Gonsebatt 2010) *"It is known that melatonin scavenges oxygen and* *nitrogen-based reactants generated in mitochondria. This limits the loss of the intramitochondrial glutathione and lowers mitochondrial protein damage, improving electron transport chain (ETC) activity and reducing mtDNA damage. Melatonin also increases the activity of the complex I and complex IV of the ETC, thereby improving mitochondrial respiration and increasing ATP synthesis under normal and stressful conditions."* (Leon et al. 2005) It also helps prevent the breakdown of the mitochondrial membrane potential, decrease electron leakage, and thereby reduce the formation of superoxide anions. (Hardeland 2005) Pharmacological doses of melatonin not only scavenge reactive oxygen and nitrogen species, but enhance levels of glutathione and the expression and activities of some glutathione-related enzymes. (Limon-Pacheco and Gonsebatt 2010; Gupta, Gupta, and Kohli 2003)

Melatonin can attenuate or prevent some EMF/RFR effects

Melatonin may have a protective effect in the setting of some EMF/RFR exposures, apparently in relation to these functions just described. EMF/RFR can impact melatonin; one example is exposure to 900-MHz microwave radiation promoted oxidation, which reduced levels of melatonin and increased creatine kinase and caspase-3 in exposed as compared to sham exposed rats (Kesari, Kumar, and Behari 2011).

Further types of adverse impacts can be seen in the next set of examples, but what is interesting is that melatonin can attenuate or prevent them. In an experiment exposing rats to MW from a GSM900 mobile phone with and without melatonin treatment to study renal impacts (Oktem et al. 2005), the untreated exposed rats showed increases of lipid peroxidation markers as reduction of the activities of superoxide dismutase, catalase and glutathione peroxidase indicating decrement in antioxidant status. However these negative effects were inhibited in the exposed rats treated with melatonin. Melatonin also inhibited the emergence of preneoplastic liver lesions in rats exposed to EMFs (Imaida et al. 2000). The development of DNA strand breaks was observed in RFR exposed rats; this DNA damage was blocked by melatonin (Lai and Singh 1997). Exposure of cultured cortical neurons to EMF led to an increase in 8-hydroxyguanine in neuronal mitochondria, a common biomarker of DNA oxidative damage, along with a reduction in the copy number of mitochondrial DNA and the levels of mitochondrial RNA transcripts; but these effects could all be prevented by pretreatment with melatonin (Xu et al. 2010). In a study of skin lesion induced by exposure to cell phone radiation, the skin changes in the irradiated group (which included thicker stratum corneum, epidermal atrophy, papillamatosis, basil cell proliferation, increased epidermal granular cell layer and capillary proliferation, impaired collagen tissue distribution and separation of collagen bundles in dermis) were prevented (except for hypergranulosis) by melatonin treatment (Ozguner et al. 2004). Melatonin as well as caffeic acid phenyethyl ester (an antioxidant) both protected against retinal oxidative stress in rates exposed long-term to mobile phone irradiation (Ozguner, Bardak, and Comlekci 2006). Nitric oxide (NO) was increased in nasal and sinus mucosa in rats after EMF exposure, with this NO possibly acting as a defense mechanism suggesting tissue damage; but this was prevented by pretreatment with melatonin (Yariktas et al. 2005). Melatonin treatment significantly prevented the increase in the MDA (malondyaldehyde, a marker of lipid peroxidation) content and XO (xanthine oxidase) activity in rat brain tissue after 40 days of exposure, but it was unable to prevent the decrease of CAT activity and increase of carbonyl group contents (Sokolovic et al. 2008).

Of note, the melatonin production of infants in isolettes in neonatal intensive care units appears to be impacted by the high ELF-EMF environment, in that when infants were removed from those exposures they showed an increase in melatonin levels (Bellieni, Tei, et al. 2012). There is an increased prevalence of ASDs in children who were born prematurely (Indredavik et al. 2010; Indredavik et al. 2008; Johnson et al. 2011; Johnson et al. 2010; Johnson and Marlow 2011; Lampi et al. 2012; Limperopoulos 2009, 2010; Limperopoulos et al. 2008; Matson, Matson, and Beighley 2011; Pinto-Martin et al. 2011). There are many potential prematurity-associated factors that could contribute to increased risk for ASDs, but electromagnetic exposure might be one of them worthy of further consideration, as it could be modified; conversely, such exposures in vulnerable infants are likely to have much broader impacts beyond reducing melatonin synthesis.

Melatonin and autism

Based on the commonality of both sleep disorders and low melatonin levels, Bourgeron (2007) proposed that synaptic and clock genes are important in ASDs, and that future studies should investigate the circadian modulation of synaptic function (Bourgeron 2007). A number of melatonin-related genetic variants have been identified as associated with ASDs. Polymorphisms, deletions and polymorphisms in the ASMT gene, which encodes the last enzyme of melatonin synthesis, have been found (Pagan et al. 2011; Jonsson et al. 2010; Melke et al. 2008), and variations have been found as well for melatonin receptor genes (Chaste et al. 2010; Pagan et al. 2011; Jonsson et al. 2010). CYP1A2 polymorphisms have been found in slow melatonin metabolisers, in whom melatonin levels are aberrant and initial response to melatonin for sleep disappeared in a few weeks (Braam et al. 2012).

Regarding melatonin status in people with ASDs, a recent meta-analysis summarized the current findings as indicating that *"1) Physiological levels of melatonin and/or melatonin derivatives are commonly below average in ASD and correlate with autistic behavior, 2) Abnormalities in melatonin-related genes may be a cause of low melatonin levels in ASD, and 3) … treatment with melatonin significantly improves sleep duration and sleep onset latency in ASD."* (Rossignol and Frye 2011) The meta-analysis also showed that polymorphisms in melatonin-related genes in ASD could contribute to lower melatonin concentrations or an altered response to melatonin, but only in a small percentage of individuals, since pertinent genes were found in only a small minority of those screened.

Autism AND Melatonin AND Glutathione

Whereas PubMed searches for “autism AND melatonin” and “autism AND glutathione” each coincidentally yielded 72 citations, and “melatonin AND glutathione” yielded 803 citations, the search for “autism AND melatonin AND glutathione” yielded zero citations. This is interesting given the strong connection of melatonin and glutathione metabolically, as discussed above, alongside of the strongly established interest in both glutathione and melatonin in ASD research and increasingly in clinical practice. Hopefully one contribution of an investigation of EMF/RFR links to ASDs will be to help bring attention to this relationship, which may help identify potential environmental and physiological causes for low melatonin in those without pertinent mutations. Of pertinence, tryptophan hydroxylase (TPH2) – the rate limiting enzyme in the synthesis of serotonin, from which melatonin is derived – is extremely vulnerable to oxidation, and tends to misfold when its cysteine residues are oxidized, with the enzyme being converted to a redox-cycling quinoprotein (Kuhn and Arthur 1999; Kuhn and Geddes 1999; Kuhn et al. 2011; Kuhn and Arthur 1997).

3. Disturbed immune function

There is by now a broad appreciation of the presence of immune disturbances in ASDs, to the point where there is an emerging discussion of ASDs as neuroimmune disorders (Bilbo, Jones, and Parker 2012; Persico, Van de Water, and Pardo 2012). Research identifying immune features in ASDs spans from genetics where risk genes have been identified to epigenetics where altered expression of immune genes is being reported as prominent in ASD epigenetics (Kong et al. 2012; Waly et al. 2012; Lintas, Sacco, and Persico 2012), and also includes prenatal infectious and immune disturbances as risk factors for autism as well as other neurodevelopmental and neuropsychiatric diseases as well as other conditions such as asthma (Patterson 2011; Smith et al. 2007; Fox, Amaral, and Van de Water 2012). Immune disturbances in infants and children with ASD are heterogeneous, with some but not all manifesting autoimmunity (Soumiya, Fukumitsu, and Furukawa 2011; Martin et al. 2008). Anecdotally, recurrent infection is common while on the other hand some get sick less often than their peers. It is common for people with autism to have family members with immune or autoimmune diseases (Croen et al. 2005). The immune system is turning out to have an important role in brain development (Bilbo and Schwarz 2012; Schwarz and Bilbo 2012; Boksa 2010). As mentioned, glial activation associated with brain immune response has been identified in a growing number of studies. Whether or not EMF/RFR contributes to these features of ASDs causally, based on the evidence below regarding immune impacts of EMF/RFR exposure (which is also reviewed much more thoroughly by Johansson in Section 8 of the present

Bioinitiative Report) (Blank 2012), it is certainly plausible that such exposures could serve as aggravating factors.

Low-intensity exposures

It is clear that the body's immune defense system responds to very low-intensity exposures. Chronic exposure to factors that increase allergic and inflammatory responses on a continuing basis is likely to be harmful to health, since the resultant chronic inflammatory responses can lead to cellular, tissue and organ damage over time. We are increasingly appreciating the extent to which many chronic diseases are related to chronic immune system dysfunction. Disturbance of the immune system by very low-intensity electromagnetic field exposure is discussed as a potential underlying cause for cellular damage and impaired healing (tissue repair), which could lead to disease and physiological impairment (Johansson 2009; Johannson 2007).

Both human and animal studies report that exposures to EMF and RFR at environmental levels associated with new technologies can be associated with large immunohistological changes in mast cells as well as other measures of immune dysfunction and dysregulation. Mast cells not only can degranulate and release irritating chemicals leading to allergic symptoms; they are also widely distributed in the body, including in the brain and the heart, which might relate to some of the symptoms commonly reported in relation to EMF/RFR exposure (such as headache, painful light sensitivity, and cardiac rhythm and palpitation problems).

Consequences of immune challenges during pregnancy

As mentioned, infection in pregnancy can also increase the risk of autism and other neurodevelopmental and neuropsychiatric disorders via maternal immune activation (MIA). Viral, bacterial and parasitic infections during pregnancy are thought to contribute to at least 30% of cases of schizophrenia (Brown and Derkits 2010). The connection of maternal infection to autism is supported epidemiologically, including in a Kaiser study where risk was associated with psoriasis and with asthma and allergy in the second trimester (Croen et al. 2005), and in a large study of autism cases in the Danish Medical registry (Atladottir et al. 2010) with infection at any point in pregnancy yielding an adjusted hazard ration of 1.14 (CI: 0.96-1.34) and when infection occurred during second trimester the odds ratio was 2.98 (CI: 1.29-7.15). In animal models, while there is much variation in study design, mediators of the immune impact appear to include oxidative stress, interleukin-6 and increased placental cytokines (Smith et al. 2007; Patterson 2009; Boksa 2010). Garbett et al. (2012) commented on several mouse models of the effects of MIA on the fetal brain that *"The overall gene expression changes suggest that the response to MIA is a neuroprotective attempt by the developing brain to counteract environmental stress, but at a cost of disrupting typical neuronal differentiation and axonal growth."* (Garbett et al. 2012). Maternal fetal brain-reactive autoantibodies have also been identified in some cases (Braunschweig et al. 2012; Braunschweig and Van de Water 2012; Fox, Amaral, and Van de Water 2012; Goines et al. 2011; Wills et al. 2009; Wills et al. 2011; Zimmerman et al. 2007).

Although we have evidence of immune impacts of EMF/RFR, the impact of repeated or chronic exposure to EMF and RFR during pregnancy is poorly studied; could this trigger similar immune responses (cytokine production) and stress protein responses, which in turn would have effects on the fetus? Although this has been poorly studied, we do have data that very low cell phone radiation exposures during both human and mouse pregnancies have resulted in altered fetal brain development leading to memory, learning, and attention problems and behavioral problems (Aldad et al. 2012).

Potential immune contributions to reactivity and variability in ASDs

Immune changes in ASDs appear to be associated with behavioral change (Shi et al. 2003; Ashwood et al. 2008; Ashwood et al. 2011; Breece et al. 2012; Heuer et al. 2008), but the mechanisms are complex and to date poorly understood (Careaga and Ashwood 2012) and likely will need to be elucidated through systems biology methods that capture multisystem influences on the interactions across behavior, brain and immune regulation (Broderick and Craddock 2012), including electrophysiology.

Two of the particularly difficult parts of ASDs are the intense reactivity and the variability in assorted symptoms such as tantrums and other difficult behaviors. Children with ASDs who also have gastrointestinal symptoms and marked fluctuation of behavioral symptoms have been shown to exhibit distinct innate immune abnormalities and transcriptional profiles of peripheral blood monocytes (Jyonouchi et al. 2011). It is worth considering EMF/RFR exposures could be operating through related mechanisms so as to add to allostatic loading in ways that exacerbate behavior. In Johansson 2006 and 2007 a foundation is provided for understanding how chronic EMF/RFR exposure can compromise immune function and sensitize a person to even small exposures in the future (Johannson 2007; Johansson et al. 2006). Johansson discusses alterations of immune function at environmental levels resulting in loss of memory and concentration, skin redness and inflammation, eczema, headache, and fatigue. Mast cells that degranulate under EMF and RFR exposures and substances secreted by them (histamine, heparin and serotonin) may contribute to features of this sensitivity to electromagnetic fields (Johansson et al. 2006). Theoharides and colleagues have argued that environmental and stress related triggers might activate mast cells, causing inflammatory compromise and leading to gut-blood-brain barrier compromise, seizures and other ASD symptoms (Theoharides et al. 2012, 2010), and that this cascade of immune response and its consequences might also be triggered in the absence of infection by mitochondrial fragments that can be released from cells in response to stimulation by IgE/anti-IgE or by the proinflammatory peptide substance P (Zhang, Asadi, et al. 2012).

Seitz et al. (2005) reviewed an extensive literature on electromagnetic hypersensitivity conditions reported to include sleep quality, dizziness, headache, skin rashes, memory and concentration impairments related to EMF and RFR (Seitz, 2005). Some of these symptoms are common in ASDs, whether or not they are due to EMF/RFR exposure, and the experience of discomfort may be hard to document due to difficulties with self-reporting in many people with ASDs.

Johansson (2007, 2009) also reports that benchmark indicators of immune system allergic and inflammatory reactions occur under exposure conditions of low-intensity non-ionizing radiation (immune cell alterations, mast cell degranulation histamine-positive mast cells in biopsies and immunoreactive dendritic immune cells) (Johannson 2007; Johansson 2009). In facial skin samples of electro- hypersensitive persons, the most common finding is a profound increase in mast cells as monitored by various mast cell markers, such as histamine, chymase and tryptase (Johansson et al. 2001). In ASDs, infant and childhood rashes, eczema and psoriasis are common, and they are common in family members as well (Bakkaloglu et al. 2008).

4. Alteration of and damage to cells in the brain

Brain cells have a variety of ways of reacting to environmental stressors, such as shape changes, metabolic alterations, upregulation or downregulation of neurotransmitters and receptors, other altered functionality, structural damage, production of un-metabolizable misfolded proteins and other cellular debris, and apoptosis; these range along a spectrum from adaptation to damage and cell death. These types of alterations can be looked at in animals under controlled conditions, but in human beings direct cellular examination can only be done on surgical biopsy tissue – which is hardly ever available in people with ASDs – or after death, at which point there has been a whole lifetime of exposures that are generally impossible to tease apart if there were even motivation to do so. This complicates the comparison of brain cell and tissue-related pathophysiology between what is seen in ASDs and what is associated with EMF/RFR exposures.

Brain cells

Impact of EMF/RFR on cells in the brain has been documented by some of the studies that have examined brain tissue after exposure, although the interpretation of inconsistencies across studies is complicated by sometimes major differences in impact attributable to differences in frequencies and duration of exposure, as well as to differences in resonance properties of tissues and other poorly understood constraints on cellular response. These studies and methodological considerations have been reviewed in depth in Belyaev, 2012 in section 15 of the 2012 BioInitiatve Report (Belyaev 2012), as well as by Salford et al. (2012) in Section 10 (Salford, Nittby, and Persson 2012). A few examples of observations after exposure have included dark neurons (an indicator of neuronal damage), as well as alteration of neuronal firing rate (Bolshakov and Alekseev

1992), and upregulation of genes related to cell death pathways in both neurons and astrocytes (Zhao, Zou, and Knapp 2007). Astrocytic changes included increased GFAP and increased glial reactivity (Chan et al. 1999; Ammari et al. 2008; Ammari et al. 2010; Brillaud, Piotrowski, and de Seze 2007), as well as astrocyte-pertinent protein expression changes detected by Fragopoulou et al, 2012 as mentioned above. Also observed has been a marked protein downregulation of the nerve growth factor glial maturation factor beta (GMF) which is considered as an intracellular signal transduction regulator in astrocytes, which could have significant impact on neuronal-glial interactions as well as brain cell differentiation and tumor development. Diminution of Purkinje cell number and density has also been observed, (Ragbetli et al. 2010) including in two studies of the impacts of perinatal exposure (Albert, 1981; Albert, 1981). Promotion of pro-inflammatory responses in EMF-stimulated microglial cells has also been documented (Yang et al. 2010).

Neuropathology findings in ASDs have been varied and have been interpreted according to various frameworks ranging from a regionalized approach oriented to identifying potential brain relationships to ASD's behavioral features (Amaral, Schumann, and Nordahl 2008) to identifying receptor, neurotransmitter and interneuron abnormalities that could account for an increased excitation/inhibition ratio (Levitt 2009; Geschwind 2007; Anney 2010; Casanova 2006; Rubenstein 2003). Studies have documented a range of abnormalities in neurons, including altered cellular packing in the limbic system, reduced dendritic arborization, and reductions in limbic GABAergic systems. Over the past decade a shift has occurred from presuming that all pertinent brain changes occurred prior to birth, to an acknowledgement that ongoing cellular processes appear to be occurring not only after birth but well into adulthood (Bauman and Kemper 2005). One of the reasons for this shift was the observation that head size (as well as brain weight and size) was on average larger in children with autism, and the head sizes of children who became diagnosed with autism increased in percentile after birth (Herbert 2005).

Neuroinflammation, glial activation and excitotoxicity

Although much attention has been paid in ASD brain literature to specific regions manifesting differences in size and activity in comparison to those without ASDs, there are other observations that are not strictly regional in nature, such as more widely distributed scaling differences (e.g. larger brains, wider brains, increased white matter volume, along with altered functional connectivity and coherence to be discussed below). Recently more studies have appeared identifying pathophysiological abnormalities such as neuroinflammation, mitochondrial dysfunction and glutathione depletion in brain tissue. Neuroinflammation was first identified in a study of postmortem samples from eleven individuals aged 5-44 who had died carrying an ASD diagnosis, in which activated astrocytes and microglial cells as well as abnormal cytokines and chemokines were found. Other research has identified further astrocyte abnormalities include, altered expression of astrocyte markers GFAP abnormalities including elevation, antibodies, and altered signaling (Laurence 2005; Singh 1997; Fatemi et al. 2008). Increased microglia activation and density as well as increased myeloid dendritic cell frequencies have also been documented. (Vargas et al. 2005; Breece et al. 2012; Tetreault et al. 2012), as has abnormal microglial-neuronal interactions (Morgan et al. 2012). Recently through use of the PET ligand PK11105 microglial activation was found to be significantly higher in multiple brain regions in young adults with ASDs (Suzuki et al. 2013). Genes associated with glial activation have been documented as upregulated. Garbett et al measured increased transcript levels of many immune genes, as well as changes in transcripts related to cell communication, differentiation, cell cycle regulation and chaperone systems (Garbett et al. 2008). Voineaugu and colleagues performed transcriptomic analysis of autistic brain and found a neuronal module of co-expressed genes which was enriched with genetically associated variants, and an immune-glial module showing no such enrichment for autism GWAS signals (Voineagu et al. 2011).

Neuroinflammation also does not appear to be strictly localized in a function-specific fashion, and it may contribute both to more broadly distributed and more focal features for tissue-based reasons. It may be that brain regions with particular prominence in ASDs may have distinctive cellular characteristics – e.g. the amygdala (Baron-Cohen et al. 2000; Dziobek et al. 2010; Hall et al. 2010; Mercadante et al. 2008; Nordahl et al. 2012; Otsuka et al. 1999; Schulkin 2007; Schumann and Amaral 2006; Schumann et al. 2009; Truitt et al. 2007; Zirlinger and Anderson 2003), which may have a larger or more reactive population of astrocytes (Johnson, Breedlove, and Jordan 2010) or the basal ganglia which may have greater sensitivity to even subtle hypoxia or perfusion abnormalities. In this case it may be the histology of these areas that makes them vulnerable to environmental irritants, and this may contribute to how environmental factors such as EMF/RFR might trigger or aggravate some of ASD's features. More widely distributed brain tissue pathology be part of what leads to differences in ASDs in brain connectivity. However these types of tissue-function relationships have been poorly investigated. The contribution of tissue differences is one of the physical considerations covered by Belyaev (2012) in Section 15 of the 2012 BioInitiative Report (Belyaev, 2012).

Various signs of mitochondrial dysfunction and oxidative stress have also been identified in the brain. Findings include downregulation of expression of mitochondrial electron transport genes (Anitha, Nakamura, Thanseem, Matsuzaki, et al. 2012) or deficit of mitochondrial electron transport chain complexes (Chauhan et al. 2011), brain region specific glutathione redox imbalance (Chauhan, Audhya, and Chauhan 2012), and evidence of oxidative damage and inflammation associated with low glutathione redox status (Rose, Melnyk, Pavliv, et al. 2012). Oxidative stress markers were measured as increased in cerebellum (Sajdel-Sulkowska, Xu, and Koibuchi 2009).

Additional support for the presence of tissue pathophysiology-based changes in brains of people with ASDs comes from the various studies documenting reduction in Purkinje cell numbers (Whitney et al. 2009; Whitney et al. 2008; Bauman and Kemper 2005; Shi et al. 2009; Blatt and Fatemi 2011; Fatemi et al. 2002; Fatemi et al. 2012), possibly due to oxidative stress and an increased excitation/inhibition ratio that could potentially be acquired (Fatemi et al. 2012). Also of note are changes in the glutamatergic and GABAergic systems, which when imbalanced can disturb the excitation/inhibition ratio and contribute to seizure disorders; reductions in GABA receptors as well as in GAD 65 and 67 proteins that catalyse the conversion of glutamate into GABA have been measured. (Yip, Soghomonian, and Blatt 2007, 2008, 2009) A consensus statement on the cerebellum in ASDs stated that, *"Points of consensus include presence of abnormal cerebellar anatomy, abnormal neurotransmitter systems, oxidative stress, cerebellar motor and cognitive deficits, and neuroinflammation in subjects with autism."* (Fatemi et al. 2012)

Some indirect corroboration for these findings has come from neuroimaging, where the initial hypothesis regarding the tissue basis of the larger size of brains in so many people with autism – that it was due to a higher density of neurons and more tightly packed axons – came under question with the emergence of contradictory findings, well reviewed a few years ago by Dager and colleagues (Dager et al. 2008). These include reduced rather than increased density of NAA (n-acetylaspartate, a marker of neuronal integrity and density that is produced in the mitochondria), reduced rather than increased fractional anisotropy suggesting less tightly packed axonal bundles (Bode et al. 2011; Cascio et al. 2012; Mak-Fan et al. 2012; Travers et al. 2012; Walker et al. 2012; Wolff et al. 2012); Sundaram, 2008) and greater rather than lower diffusivity, all of which may be more consistent with lower density of tissue and tissue metabolites and more fluid, which could be consistent with neuroinflammation and/or oxidative stress. The early postnatal development of such lower fractional anisotropy and increased diffusivity was measured in the process of occurring recently, in the first large prospective longitudinal imaging study of infants, who trended from 6 months to 2 years in the direction of these findings becoming more pronounced – but still with substantial overlap with those infants who did not develop autism (Wolff et al. 2012). This trend was consistent with prior studies showing increase in head size after birth, and added some information about what was happening in the brain to drive this size increase, although due to its methods it could only indirectly address the possibilty that emergence during the first few years of life of tissue pathophysiology disturbances such as neuroinflammation might be contributing to these trends (Herbert 2012).

There is also substantial variability across many different types of brain findings. Of interest is that a number of functional brain imaging and electrophysiology studies have identified greater heterogeneity in response to stimuli between individuals in the ASD group than individuals in the neurotypical control group (Muller et al. 2003; Dinstein et al. 2012). This may make more sense from the point of view of non-linear response – i.e. a disproportionality between output and input (as well as state and context sensitivity), in a pathophysiologically perturbed brain system. Nonlinearity has also been a significant methodological issue in EMF/RFR research because linear methods of study design and data analysis have often been insensitive to effects, whereas nonlinear methods have been argued to show greater sensitivity (Carrubba and Marino 2008; Marino, Wolcott, Chervenak, Jourd'heuil, Nilsen, Frilot, et al. 2001; Marino and Frilot 2003; Carrubba et al. 2006; Carrubba et al. 2012; Marino, Nilsen, and Frilot 2003; Marino, Wolcott, et al. 2001, 2001; Carrubba et al. 2007; Marino et al. 2000; Bachmann, 2005).

The presence of various types of tissue pathophysiology both in findings in postmortem tissue from individuals with ASDs and in documented impacts of EMF/RFR exposure are intriguing and suggest overlap in processes involved. But it is not really possible to infer any specific agent of injury from cellular responses since for the most part these are not specific but rather are stress or repair responses generic to a variety of triggers. It is important to entertain how environmental agents could contribute to brain changes in ASDs, and how these changes may develop over progress over time after the earliest periods in brain development. EMF/RFR exposures could be preconceptional, prenatal or postnatal – or all of the above; it is conceivable that this could be the case in ASDs as well.

Altered development

There is some evidence for altered brain and organism development in relation to EMF/RFR exposure. Aldad et al. 2012 exposed mice in utero to cellular telephone radiation, with resultant aberrant miniature excitatory postsynaptic currents, dose-responsive impaired glutamatergic synaptic transmission onto layer V pyramidal neurons of the prefrontal cortex (Aldad et al. 2012). Lahijani exposed preincubated chicken embryos to 50 Hz EMFs, and made the following morphological observations: *"exencephalic embryos, embryos with asymmetrical faces, crossed beak, shorter upper beak, deformed hind limbs, gastroschesis, anophthalmia, and microphthalmia. H&E and reticulin stainings, TEMS, and SEMs studies indicated EMFs would create hepatocytes with fibrotic bands, severe steatohepatitis, vacuolizations, swollen and extremely electron-dense mitochondria, reduced invisible cristae, crystalized mitochondria with degenerated cristae, myelin-like figures, macrophages engulfing adjacent cells, dentated nuclei, nuclei with irregular envelopes, degenerated hepatocytes, abnormal lipid accumulations, lipid droplets pushing hepatocytes' nuclei to the corner of the cells, abundant cellular infiltrations cellular infiltrations inside sinusoid and around central veins, disrupted reticulin plexus, and release of chromatin into cytosol,, with partially regular water layers,"* and attributed cell damage to elevated free radical induced cell membrane disruptions (Lahijani, Tehrani, and Sabouri 2009).

Although it is of great interest to characterize the changes in development associated with ASDs, it is also difficult to do in human beings because at present diagnosis is not possible until at least 2-3 years after birth. By now there have been a lot of prospective studies of infants at high risk for autism, but the in vivo brain imaging and electrophysiology data from these studies is only starting to be published, and so the for now the main sources of information are still inference backwards from post-mortem or imaging data, and animal models, both of which have clear limitations. Thus it is impossible to seek precise parallels here between what we know about the development of ASDs compared with the impacts of EMF/RFR exposures.

Nevertheless it is of real concern that such exposures have elicited some of the brain tissue changes that have been documented, both in early development and subsequently. Already noted above is the question of whether high exposures of neonates to monitoring equipment may affect the melatonin levels of neonates (Bellieni, Tei, et al. 2012); these exposures also impact heartrate variability . There are no studies yet on infants exposed to baby surveillance monitors or DECT wireless phones. However there are good laboratory testing studies yielding actual measurements of these devices that conclude: "*Maximum incident field exposures at 1m can significantly exceed those of base stations (typically 0.1 - 1 V/m). At very close distances the derived or reference exposure limits are violated" for baby surveillance monitors and DECT phones.* Further, the authors conclude that, based on very strictly controlled laboratory testing of everyday devices like baby monitors and some cordless phones *"(W)orse case peak spatial SAR values are close to the limit for the public or uncontrolled environments, e.g., IEEE802.11b and Bluetooth Class I".*(Kuhn et al. 2012) Even exposure of the fetus to laptop computer wireless emissions through the pregnant mother's use of them may on her lap involve induction of strong intracorporeal electric current densities from the power supply possibly even more than the device itself (Bellieni, Pinto, et al. 2012).

Brain blood flow and metabolism

Cerebral perfusion and metabolism abnormalities have been identified in close to 2 dozen papers studying autistic cohorts. Cerebral perfusion refers to the quantity of blood flow in the brain. Abnormal regulation of cerebral perfusion is found in a range of severe medical conditions including tumors, vascular disease and epilepsy. Cerebral hypoperfusion has also been found in a range of psychiatric disorders (Theberge 2008). Neurocognitive hypotheses and conclusions, as well as localization of perfusion changes, have been heterogeneous across these papers. Hypoperfusion or diminished metabolism has been identified in frontal regions (George, 1992; Gupta, 2009; Degirmenci, 2008; Wilcox, 2002; Galuska, 2002; Ohnishi, 2000; temporal lobes (Boddaert, 2002 ; Burroni, 2008 ; Degirmenci, 2008, Galuska, 2002, George, 1992 ; Hashimoto, 2000, Ohnishi, 2000, Ryu, 1999, Starkstein, 2000, Zilbovicius, 2000), as well as a variety of subcortical regions including basal ganglia (Degirmenci, 2008; Ryu, 1999; Starkstein, 2000), cerebellum (Ryu, 1999), limbic structures (Ito, 2005, Ohnishi, 2000) and thalamus (Ito, 2005, Ryu, 1999, Starkstein, 2000) – i.e., in a widely distributed set of brain regions. It is interesting to note that even with this regional variation in localization, most of these publications showed that cerebral perfusion was *reduced*; in the only one of those studies reporting some areas of localized hyperfusion, these areas were found in the middle of areas in the frontal pole and temporal lobe that were hypoperfused (McKelvey 1995), Only one study showed no difference in perfusion between autistic and control subjects (Herold 1988). Possibly because virtually all of these studies were oriented toward testing neuropsychological rather than pathophysiological hypotheses, there were no probes or tests reported to unearth the tissue level alterations that might be underlying these reductions in blood flow in these brains.

While a large number of animal studies have documented BBB abnormalities from EMF/RFR exposures, only a few PET studies have been performed evaluating EMF exposure effects upon brain glucose metabolism. Volkow et al. performed PET scans both with and without EMF exposure (50 min of GSM-900 with maximum SAR of 0.901 W/kg), and the participants were blinded to the exposure situation (Volkow et al. 2011). A 7% increase in metabolism in the exposure situation compared to controls was identified regionally on the same side of the head as where the mobile phone was placed, in the right orbitofrontal cortex and in the lower part of the right superior temporal gyrus . The strength of the E-field from the phones correlated positively with the brain activation, which the authors hypothesized was from an increase in brain neuron excitability. . A subsequent smaller study by Kwon et al. demonstrated not increased but decreased brain ^{18}FDG uptake after GSM-900 exposure, this time in the temporoparietal junction (Kwon et al. 2011).

Many possible mechanisms could be involved in the metabolic and perfusion abnormalities identified, ranging from altered neuronal activity that was hypothesized in the Volkow et al. (2011) ^{8}FDG PET study to narrowing of vascular lumen in the setting of reduced perfusion. Underlying tissue pathophysiology-based phenomena could influence the measurable metabolism and perfusion abnormalities, via mechanisms such as excitotoxicity, cell stress response, constriction of capillary lumen by activated astrocytes, volume effects of vascular extravasation, subtle alterations in blood viscosity due to immune or oxidative stress-associated blood chemical changes, with other possibilities as well. Given the types of damage at the cellular level covered in this pathophysiology section so far – including oxidative stress, membrane and barrier function damage and poorly functioning channels, which occur both in ASDs as a consequence of EMF/RFR exposure, and given the heterogeneity of localization of abnormalities in the autism perfusion papers as well as considerations of nonlinearity, it may not be so surprising that the results in the two PET studies of human impacts of EMF exposure were not consistent.

6. Electrophysiology perturbations

At this stage the argument we hit a key pivot point, where we look at how the alterations in molecular, cellular and systems physiological function, which occur in the brain as well as in the body, impact the transduction into the electrical signaling activities of the brain and nervous system. Certainly the cells and tissues whose physiological challenges we have already discussed provide the material substrate for the electrical activity. Although ASD behaviors are influenced by many factors, they must in principle be mediated through nervous system electrophysiology.

If the cells responsible for generating synapses and oscillatory signaling are laboring under cellular and oxidative stress, lipid peroxidation, impaired calcium and other signaling system abnormalities, then mitochondrial metabolism will fall short, all the more so because of the challenges from the immune system which in turn be triggered to a major extent by environment. How well will synapses be generated? How well will immune-activated and thereby distracted glial cells be able to modulate synaptic and network activity? (Tasker et al. 2012; Eroglu and Barres 2010; Bilbo and Schwarz 2009; Fields 2006)

At present we are in the early stages of being able to formulate these questions well enough to address them. We do know that microglial activation can impact excitatory neurotransmission mediated by astrocytes (Pascual et al. 2012). We do know that the cortical innate immune response increases local neuronal excitability and can lead to seizures (Rodgers et al. 2009; Gardoni et al. 2011). We do know that inflammation can play an important role in epilepsy (Vezzani et al. 2011). We know less about lower levels of chronic or acute pathophysiological dysfunction and how they may modulate and alter the brain's electrophysiology.

Seizures and Epilepsy

EEG signals in ASDs are abnormal on a variety of levels. At the most severe level, EEGs show seizure activity. In addition to the association of some severe epilepsy syndromes (e.g. Landau Kleffner, tuberous sclerosis) with autism, the risk of epilepsy is substantially higher in people with ASDs than in the general population, with a large subset of these individuals experiencing seizure onset around puberty, likely in relation to aberrations in the dramatic and brain-impactful hormonal shifts of that phase of life. Although less than 50% of people clearly have seizures or epilepsy, a much larger number have indications of epileptiform activity, and an even larger percent have subclinical features that can be noted by a clinical epileptologist though not necessarily flagged as of clinical concern.

Epileptic seizures can be both caused by and cause oxidative stress and mitochondrial dysfunction. Seizures can cause extravasation of plasma into brain parenchyma (Mihaly and Bozoky 1984; Librizzi et al. 2012; Marchi et al. 2010; van Vliet et al. 2007; Yan et al. 2005) which can trigger a vicious circle of tissue damage from albumin and greater irritability, as discussed above. Evidence suggests that if a BBB is already disrupted, there will be greater sensitivity to EMF/RFR exposure than if the BBB were intact (Tore et al. 2002; Tore et al. 2001), suggesting that such exposures can further exacerbate vicious circles already underway.

The combination of pathophysiological and electrophysiological vulnerabilities has been explored in relation to the impact of EMF/RFR on people with epilepsy – which, as discussed above, is a lot more common in ASDs than in the general population.. EMF/RFR exposures from mobile phone emissions have been shown to modulate brain excitability and to increase interhemispheric functional coupling (Vecchio et al. 2012; Tombini et al. 2012). In a rat model the combination of picrotoxin and microwave exposure at mobile phone-like intensities led to a progressive increase in neuronal activation and glial reactivity, with regional variability in the fall-off of these responses three days after picrotoxin treatment (Carballo-Quintas et al. 2011), suggesting a potential for interaction between a hyperexcitable brain and EMF/RFR exposure.

One critical issue here is nonlinearity and context and parameter sensitivity of impact. In one study, rat brain slices exposed to EMF/RFR showed reduced synaptic activity and diminution of amplitude of evoked potentials, while whole body exposure to rats led to synaptic facilitation and increased seizure susceptibility in the subsequent analysis of neocortical slices (Varro et al. 2009). Another study unexpectedly identified enhanced rat pup post-seizure mortality after perinatal exposure to a specific frequency and intensity of exposure, and concluded that apparently innocuous exposures during early development might lead to vulnerability to stimuli presented later in development (St-Pierre et al. 2007)

Sleep

Sleep involves a profound change in brain electrophysiological activity, and EEG abnormalities including disrupted sleep architecture figure in sleep challenges in ASD. Sleep symptoms include bedtime resistance, sleep onset delay, sleep duration and night wakings, and sleep architecture can involve significantly less efficient sleep, less total sleep time, prolonged sleep latency, and prolonged REM latency (Buckley et al. 2010; Giannotti et al. 2011), with these sleep problems being worse in children with ASDs who regressed than in those who did not regress into their autism (Giannotti, 2011). EEG abnormalities have also been associated with EMF/RFR exposure, including disrupted sleep architecture as well as changes in sleep spindles and in the coherence and correlation across sleep stages and power bands during sleep (Borbely, 1999; Huber, 2003).

Sleep disturbance symptoms are also common in both situations. Insomnia is commonly reported in people who are chronically exposed to low-level wireless antenna emissions. Mann (1996) reported an 18% reduction in REM sleep, which is key to memory and learning functions in humans. In ASDs sleep difficulties are highly pervasive and disruptive not only to the affected individual but also to their whole family due to the associated problems such as noise and the need for vigilance.

The multileveled interconnections involved in the modulation of sleep exemplify the interconnectedness of the many levels of pathophysiology reviewed here: *"Extracellular ATP associated with neuro- and glio-transmission, acting via purine type 2 receptors, e.g., the P2X7 receptor, has a role in glia release of IL1 and TNF. These substances in turn act on neurons to change their intrinsic membrane properties and sensitivities to neurotransmitters and neuromodulators such as adenosine, glutamate and GABA. These actions change the network input-output properties, i.e., a state shift for the network."* (Clinton et al. 2011) With disturbance simultaneously at so many of these levels, it is not surprising that sleep dysregulation is nearly universal in ASDs, and common in the setting of EMF/RFR exposures.

Quantitative electrophysiology

While clinical reading of EEG studies is done visually, a growing number of studies are examining EEG and MEG data using digital signal processing analysis, and often using data collected in controlled research settings with high density array equipment and carefully designed stimuli paradigms. In these settings a variety of abnormalities have been identified other than epileptic. These include abnormalities in the power spectrum, i.e. the distribution of power over the different frequencies present, with some studies showing impaired or reduced gamma-and activity (Sun et al. 2012; Rojas et al. 2008; Rippon, 2007) and others 8) showing reduction of spectral power across all bands (Tierney et al. 2012) while still others showed increased high-frequency oscillations. (Orekhova et al. 2007) Abnormalities in coherence and synchronization between various parts of the brain have been found (Muller 2008; Muller et al. 2011; Wass 2011), comparable to abnormal functional connectivity measured by fMRI (Just et al. 2004) but measurable using EEG or MEG with higher temporal resolution (Duffy, 2012; Isler, 2010; Murias, 2007; Murias, 2007; Coben, 2008). Several studies have identified reduced complexity and increased randomness in EEGs of people with autism (Lai et al. 2010; Catarino et al. 2011), as well as an increase in power but a reduction in coherence (Isler et al. 2010; Mathewson et al. 2012). Some electrophysiological metrics are emerging as potential discriminators between brain signal from individuals with ASDs and those who are neurotypical, such as a wavelet-chaos-neural network methodology applied to EEG signal (Ahmadlou, Adeli, and Adeli 2010).

EMF/RFR also has impacts at levels of brain function measurable by these techniques. At various frequencies and durations of exposure it has been noted to impact alpha and beta rhythms (Hinrikus et al. 2008), to increase randomness (Marino, Nilsen, and Frilot 2003; Marino and Carrubba 2009), to alter power, to modulate interhemispheric synchronization (Vecchio et al. 2007), to alter electrical activity in brain slices (Tattersall et al. 2001) and to alter the patterns of coordination (spectral power coherence) across the major power bands (Hountala et al. 2008). Bachman et al. (2006) showed statistically significant changes in EEG rhythms and dymanics occurred in between 12% and 20% of healthy volunteers (Bachmann, 2006). In children, exposures to cell phone radiation have resulted in changes in brain oscillatory activity during some memory tasks.

Sensory processing

At the symptomatic level issues with sensory processing are highly prevalent in ASDs. Phenomenology can include hypersensitivity to external stimuli, hyposensitivity to internal sensations and difficulty localizing sensation including pain, and difficulty processing more than one sensory channel at one time. (Robledo, Donnellan, and Strandt-Conroy 2012; Perry et al. 2007; Sacco et al. 2010) There is now electrophysiological evidence of abnormalities at early (brainstem) stages of sensory processing, as well as in later stages of processing that occur in the cortex. Some studies have shown lower and some longer latencies of response to an auditory stimulus. Domains of perception where the performance of people with ASDs is superior to that of neurotypical individuals have been identified. (Marco et al. 2011) *"It is obvious…that sensory processing abnormalities in ASD are distributed rather than localized; sensory abnormalities in ASD obviously span multiple dimensions of latency, adaptation, magnitude and behavior abnormalities, with both enhanced and impaired behavior associated with aberrant cortical responses. Given this diversity in findings, the heterogeneity of ASD, and broad variability seen over and over again in the ASD groups almost irrespective of the study, it is hard to imagine that one single theory could account for all of these observations…. It is therefore probable that several mechanisms and neuronal abnormalities, most likely at multiple levels (from single neurons through to inter-area connections), all contribute to varying degrees to the abnormal sensory processing observed in ASD. It is also likely that no single mechanism is unique to one sensory modality, which is why we see such a widely distributed range of abnormalities across modalities."* (Kenet 2011)

It is also possible that the mechanisms may not simply be neural – they may also be modulated by glial, metabolic, immune, perfusional and other physiological processes and physical properties as well. Yet although there is some consideration of the pathophysiology-sensory function interaction (Kern et al. 2010), it has basically not been fleshed out in studies of ASDs with experimental designs integrating pathophysiological and electrophysiology.

Kenet et al. (2010) demonstrated environmental vulnerability of sensory processing in the brain by the exposure of rat dams to noncoplanar polychlorinated biphenyls (PCBs), during gestation and for three subsequent weeks of nursing (Kenet, 2011). *"Although the hearing sensitivity and brainstem auditory responses of pups were normal, exposure resulted in the abnormal development of the primary auditory cortex (A1). A1 was irregularly shaped and marked by internal nonresponsive zones, its topographic organization was grossly abnormal or reversed in about half of the exposed pups, the balance of neuronal inhibition to excitation for A1 neurons was disturbed, and the critical period plasticity that underlies normal postnatal auditory system development was significantly altered. These findings demonstrate that developmental exposure to this class of environmental contaminant alters cortical development."* (Kenet et al. 2007). This study may be particularly relevant for EMF/RFR exposures, as the noncoplanar PCBs were discussed above as targeting calcium signaling as do EMF/RFR exposures – i.e. they both converge upon a common cellular mechanism (Pessah and Lein 2008; Stamou et al. 2012), justifying exploring the hypothesis that the outcomes one might expect from EMF/RFR could be similar.

Autonomic dysregulation

Although there are a fair number of negative studies regarding the impact of EMF/RFR exposure on the autonomic nervous system, increased HRV and autonomic disturbances have been documented (Andrzejak et al. 2008; Szmigielski et al. 1998; Bortkiewicz et al. 2006; Graham et al. 2000; Saunders and Jefferys 2007). Buchner and Eger (2010), in a study in rural Germany of the health impacts of exposures from a new base station yielding novel exposure to EMF/RFR, saw a significant elevation of the stress hormones adrenaline and noradrenaline during the first six months with a concomitant drop in dopamine, with a failure to restore the prior levels after a year and a half. These impacts were felt by the young, the old and the chronically ill, but not by healthy adults (Buchner and Eger 2011).

Effects on the neonate are also evident. Bellieni et al (2008) found that heart rate variability is adversely affected in infants hospitalized in isolettes or incubators where ELF-EMF levels are in the 0.8 to 0.9 µT range (8 to 9 mG). Infants suffer adverse changes in heart rate variability, similar to adults (Bellieni et al. 2008). This electromagnetic stress may have lifelong developmental impacts, based on a study showing that in utero beta 2 agonist exposure can potentially induce a permanent shift in the balance of sympathetic-to-parasympathetic tone (Witter et al. 2009).

Meanwhile clinical observation and a growing body of literature support a major role for stress in ASDs (Anderson and Colombo 2009; Anderson, Colombo, and Unruh 2012; Daluwatte et al. 2012; Ming et al. 2011), with variability amongst individuals in the severity of the stress response but a tendency to have high tonic sympathetic arousal at baseline (Hirstein, Iversen, and Ramachandran 2001; Toichi and Kamio 2003; Ming, Julu, et al. 2005; Mathewson et al. 2011; Cheshire 2012; Chang et al. 2012).

The impact of EMF/RFR exposure can also be greatly influenced by the stress system status of the individual being exposed. Tore et al sympathecotomized some of his rats before exposure to GSM, to simulate cell phone exposure (Tore et al. 2002; Tore et al. 2001). Salford et al. (2012) reviewed the results:

> *"Comparing the animals, which had been subjected to ganglionectomy, to the other animals, Töre et al. made an interesting observation: as expected, albumin extravasation was more prominent in the sympathectomised sham-exposed rats as compared to normal exposed rats. This was due to the fact that the sympathectomised rats were in a chronic inflammation-prone state with hyper-development of pro-inflammatory structures, such as the parasympathetic and sensory inputs as well as mast cells, and changes in the structure of the blood vessels. Such an inflammation-prone state has a well-known effect on the BBB leakage. However, when comparing sham-exposed sympathectomised rats to GSM-exposed sympathectomised rats, a remarkable increase in albumin leakage was present in the GSM exposed sympathectomised rats compared to the sham rats.* ***In the GSM-exposed sympathectomised rats, both brain areas and the dura mater showed levels of albumin leakage resembling those observed in positive controls after osmotic shock****.[emphasis added] Indeed, more attention should be paid to this finding, since it implicates that the sensitivity to EMF-induced BBB permeability depends not only on power densities and exposure modulations, but also on the initial state of health of the exposed subject."* (Salford, Nittby, and Persson 2012)

This dramatically greater impact on an autonomically and immunologically vulnerable set of animals raises concerns since the vulnerabilities of these animals bear some resemblance to the pathophysiological challenges of individuals with ASDs.

The interconnection between stress and brain connectivity (or coherence) in ASDs is brought out by Narayanan et al. (2010) n a pilot study testing the impact of the beta blocker propranolol on brain functional connectivity measured using functional MRI (Narayanan et al. 2010). A fairly immediate increase in functional connectivity was noted from propranolol – but not from nadolol which has the same vascular effects but does not cross the BBB. Propranolol decreases the burden of norepinephrine, thereby reducing the impact of stress systems on brain processing, and the authors interpreted these effects as creating an improvement of the brain's signal-to-noise ratio (Hasselmo, 1997), allowing it to utilize and coordinate more remote parts of its networks. This suggests that stressors such as EMF/RFR, by adding non-biologically meaningful noise to the system, might have the opposite effects, degrading coherent integration.

C. De-tuning of the Brain and Organism

1. Electromagnetic signaling, oscillation and synchrony are fundamental, and vulnerable

While electrophysiological activity is an intrinsic property of the nervous system, electromagnetic signaling are vital parts of every cell and of molecular structure.

> *"All life on earth has evolved in a sea of natural low-frequency electromagnetic (EM) fields. They originate in terrestrial and extraterrestrial sources. The ever-growing use of electric power over the last century has sharply modified this natural environment in urban environments. Exposure to power-frequency fields far stronger than the natural environment is now universal in civilized society."* (Adey 1994)

Adey published some of the earliest scientific studies on the "cooperativity" actions of cells in communication. Studies showing us that the flux of calcium in brain tissue and immune cells is sensitive to ELF-modulated radiofrequency fields is actually telling us that some of the most fundamental properties of cells and thus of our existence can be modulated by EMF/RFR.

> *"…in first detection of environmental EM fields in tissues, there appears to be a general consensus that the site of field action is at cell membranes. Strands of protein are strategically located on the surface of cells in tissue, where they act as detectors of electrical and chemical messages arriving at cell surfaces, transducing them and transmitting them to the cell interior. The structural basis for this transductive coupling by these protein strands is well known. Through them, cell membranes perform a triple role, in* ***signal detection, signal amplification, and signal transduction to the cell interior****."* (Adey 1994)

Communication between cells through gap junctions, which is a means of "metabolic cooperation," is also vulnerable to disruption, as discussed earlier.

Oscillation is also a universal phenomenon, and biological systems of the heart, brain and gut are dependent on the cooperative actions of cells that function according to principles of non-linear, coupled biological oscillations for their synchrony, and are dependent on exquisitely timed cues from the environment at vanishingly small levels (Buzsaki 2006; Strogatz 2003). The key to synchronization is the joint actions of cells that co-operate electrically - linking populations of biological oscillators that couple together in large arrays and synchronize spontaneously according to the mathematics described for Josephson junctions (Brian Josephson, the 1993 Nobel prize winner for this concept). This concept has been professionally presented in journal articles and also popularized in a book by Prof. Steven Strogatz, a mathematician at Cornell University who has written about ‘sync’ as a fundamental organizing principle for biological systems (Strogatz 2001) (Strogatz 2003).

> *“Organisms are biochemically dynamic. They are continuously subjected to time-varying conditions in the form of both extrinsic driving from the environment and intrinsic rhythms generated by specialized cellular clocks within the organism itself. Relevant examples of the latter are the cardiac pacemaker located at the sinoatrial node in mammalian hearts and the circadian clock residing at the suprachiasmatic nuclei in mammalian brains. These rhythm generators are composed of thousands of clock cells that are intrinsically diverse but nevertheless manage to function in a coherent oscillatory state. This is the case, for instance, of the circadian oscillations exhibited by the suprachiasmatic nuclei, the period of which is known to be determined by the mean period of the individual neurons making up the circadian clock. The mechanisms by which this collective behavior arises remain to be understood.”* (Strogatz 2003)

The brain contains a population of oscillators with distributed natural frequencies, which pull one another into synchrony (the circadian pacemaker cells). Strogatz has addressed the unifying mathematics of biological cycles and external factors disrupt these cycles. This also applies to mitochondria:

> *“Organisation of mitochondrial metabolism is a quintessential example of a complex dissipative system which can display dynamic instabilities. Several findings have indicated that the conditions inducing instabilities are within the physiological range and that mild perturbations could elicit oscillations. Different mathematical models have been put forth in order to explain the genesis of oscillations in energy metabolism. One model considers mitochondria as an organised network of oscillators and indicates that communication between mitochondria involves mitochondrial reactive oxygen species (ROS) production acting as synchronisers of the energy status of the whole population of mitochondria. An alternative model proposes that extramitochondrial pH variations could lead to mitochondrial oscillations.”* (Iotti, Borsari, and Bendahan 2010)

The field of bioelectromagnetics has studied exposure to very low levels of electromagnetic frequencies.

These exposures can alter critical properties of chemical reactions. *“In a chemical reaction, the bond breaks and each partner reclaims its electron from the bond, moving away to encounter a new partner. It is now an unattached, highly reactive free radical. Reforming a bond requires a meeting between two radicals with opposite electron spins, the union producing a singlet pair. The lifetime of free radicals is typically short, in the* *range of microseconds to nanoseconds. It is in this brief period that imposed magnetic fields may alter the rate and amount of product of a chemical reaction. Since the effect is only on the kinetics of chemical reactions, they are known as magnetokinetic effects (Steiner and Ulrich, 1989). They occur only in nonthermal states of biomolecular systems, defined as an insensitivity to random thermal interactions during the brief period of their existence (Walleczek, 1994). They are a consequence of a coherent quantum-mechanical step which accompanies free radical formation*." (Adey 1994)

Not just chemical reactions but synchronous biological oscillations in cells (pacemaker cells) can be disturbed and disrupted by artificial, exogenous environmental signals, whicn can lead to desynchronization of neural activity that regulates critical functions (including metabolism) in the brain, gut and heart and circadian rhythms governing sleep and hormone cycles (Strogatz, 1987). Buzsaki in his book *Rhythms of the Brain* (2006) says "*rhythms can be altered by a wide variety of agents and that these perturbations must seriously alter brain performance."* (Buzsaki 2006)

Disturbance can get increasingly disruptive as more damage occurs and more systems are thrown out of kilter and out of cooperativity. One can think of the kindling model in which repeated induction of seizures leads to longer and more sever seizures and greater behavioral involvement. The combination of disruptive and stimulatory effects of biologically inappropriate EMF/RFR exposures could contribute to disruption of synchronized oscillation and cooperativity at a myriad of levels but particularly in the brain, and this may contribute to the loss of coherence and complexity in the brain in autism, as well as dysregulation of multiple other bodily systems. Strogatz points out that there are many more ways of being desynchronized than being synchronized (Strogatz, 2003). It has even been suggested that autism itself could be due to brain desynchronization (Welsh, 2005).

2. Behavior as an "emergent property"

Although from a pathophysiological point of view one might hypothesize that a brain with greater indications of oxidative stress along with immune activation and mitochondrial dysfunction might generate different oscillatory activity than a brain in which those pathophysiological features were absent, to date almost no attention has been paid to testing this hypothesis in ASD or neurodevelopmental and neuropsychiatric conditions more generally. From this vantage point it would make sense to propose that the compromised whole body health status of at least many with ASDs would make it harder for them to maintain the resilience of their brain cells and brain activities in the face of potentially disruptive exposures. Yet the investigation of how this might occur remains a largely unexplored frontier. But from the point of view of making sense of the brain impact of environmental challenges – including but not limited to EMF-RFR – this investigation is crucial.

The pathophysiological perspective that guides this review would suggest a move away from considering the behavioral manifestations of ASDs as core 'traits,' *Instead behaviors may be better understood as 'outputs' or emergent properties – what the brain and body produce – when their physiological attributes are altered* in these fashions for whatever reasons – be they genetic, environmental or many combinations of both (Anderson 2009, 2008; Sieb 2004; Smith and Thelen 2003; Custodio et al. 2007; Herbert 2012). Sleep and consciousness have also been considered 'emergent properties' (Krueger et al. 2008; Krueger and Obal 2003). Brain oscillatory activity is critical for organizing behavior, and it arises from cells and subcellular features that are shaped by the environment and can act differently based on their functional status as well as on account of external sensory or psychosocial stimuli.

In particular, a) brain oscillatory activity is intimately connected with underlying cellular, metabolic and immune status, b) EMF/RFR is capable of perpetrating changes at each of these levels, and c) problems at each of these levels can make other problems worse. And as mentioned earlier, EMF/RFR and various toxicants can co-potentiate damage (Juutilainen and Kumlin 2006; Juutilainen, Kumlin, and Naarala 2006; Verschaeve et al. 2006; Ahlbom et al. 2008; Hoyto et al. 2008; Juutilainen 2008; Luukkonen et al. 2009; Markkanen, Juutilainen, and Naarala 2008), amplifying allostatic load.

Put together, all of this implies that the combination of these EMF/RFR impacts may quite plausibly significantly contribute both to how ASDs happen in individuals and to why there are more reported cases of ASDs than ever before (with studies showing that not all of this increase can be written off as artifact (King and Bearman 2009; Hertz-Picciotto and Delwiche 2009).

The hopeful side of this framing of the problem comes from moving beyond the increasingly anachronistic idea that autism is determined overwhelmingly by genetic code about which we can do little or nothing. An emerging model that explains much more of what we now know frames ASDs as the dynamic, active outcomes of perturbed physiological processes – again, more like a chronic but changeable 'state' than a 'trait.' In the latter model, one is empowered to strongly reduce exposures and to make aggressive constructive environmental changes – particularly in diet and nutrition, given their protective potency discussed above (Herbert and Weintraub 2012). In this way allostatic load can be reduced, physiological damage can be repaired, homeostasis can be restored and resilience and optimal function can be promoted.

III. IMPLICATIONS

A. Summary

In the above review, the case has been made that ASDs involve physiological challenges at multiple levels, and that these challenges are paralleled in the physiological impacts of EMF/RFR exposure. Evidence has also been presented to suggest that the many levels of damage and degradation of physiological and functional integrity are profoundly related to each other. Although autism spectrum disorders (ASDs) are defined by problems with behavior, communication, social interaction and sensory processing, under the surface they also involve a range of disturbances of underlying biology that find striking parallels in the physiological impacts of electromagnetic frequency and radiofrequency exposures (EMF/RFR). At the cellular and molecular level many studies of people with ASDs have identified oxidative stress and evidence of free radical damage, evidence of cellular stress proteins, as well as deficiencies of antioxidants such as glutathione. Elevated intracellular calcium in ASDs can be associated with genetic mutations but more often may be downstream of inflammation or chemical exposures. Cell membrane lipids may be peroxidized, mitochondria may function poorly, and immune system disturbances of various kinds are common. Brain oxidative stress and inflammation as well as measures consistent with blood-brain barrier and brain perfusion compromise have been documented. Changes in brain and autonomic nervous system electrophysiology can be measured and seizures are far more common than in the population at large. Sleep disruption and high levels of stress are close to universal. In parallel, all of these phenomena have also been documented to result from or be modulated by EMF/RFR exposure. Moreover, some people with ASDs have de novo mutations (that their parents do not have), and EMF/RFR exposures could contribute to this due to their potential genotoxicity. EMF/RFR exposure during pregnancy may send spurious signals to developing brain cells during pregnancy, altering brain development during critical periods, and may increase oxidative stress and immune reactivity that can increase risk for later developmental impairments, with further disruption later in development increasing risk, physiological dysregulation and severity of outcome.

All of this does not prove that EMF/RFR exposures cause autism, but it does raise concerns that they could contribute by increasing risk, and by making challenging biological problems and symptoms worse in these vulnerable individuals. Placed alongside the dramatic rise in reported cases of ASDs, that parallels the dramatic rise in deployment of wireless technologies, a strong case can be made for aggressively investigating links between ASDs and EMR/RFR, and minimizing exposures for people with autism as well as families preconceptionally, during pregnancy, and around infants and children at home, at school, and in health care centers and hospitals.

These arguments have implications for how we understand what ASDs 'are' and how they work. These implications call upon us to take the environmental contribution very seriously, which involves on the one hand a sobering appreciation of the vast array of exposures that can contribute to risk via perturbed development and physiological degradation, and on the other hand a sense that there are powerful things we can do to improve the situation.

B. Exposures and their Implications

Several thousand scientific studies over four decades point to serious biological effects and health harm from EMF and RFR as are intensively reviewed in the many detailed sections of this BioInitiative Report. These studies report genotoxicity, single-and double-strand DNA damage, chromatin condensation, loss of DNA repair capacity in human stem cells, reduction in free-radical scavengers (particularly melatonin), abnormal gene transcription, neurotoxicity, carcinogenicity, damage to sperm morphology and function, effects on behavior, and effects on brain development in the fetus of human mothers that use cell phones during pregnancy. Cell phone exposure has been linked to altered fetal brain development and ADHD-like behavior in the offspring of pregnant mice.

1. Exposures have outpaced precautions

There is no question that huge new exposures to EMF/RFRs have occurred over the past few decades. As discussed extensively in other parts of this Bioinitiative 2012 update (Sage, 2012), there is much concern that regulations to date have been based on a very limited sense of the pertinent biology, and particularly that limiting concern to thermal impacts is no longer valid since there is a wealth of evidence by now that non-thermal impacts can be biologically very powerful.

Only in the last two decades have exposures to RFR and wireless technologies become so widespread as to affect virtually every living space, and affect every member of societies around the world. Even as some disease patterns like brain tumors from cell phone use have become 'epidemiologially visible', there are no comprehensive and systematic global health surveillance programs that really keep up to report EMF/RFR health trends (Fragopoulou et al. 2010).

> "*The deployment of new technologies is running ahead of any reasonable estimation of possible health impacts and estimates of probabilities, let alone a solid assessment of risk. However, what has been missing with regard to EMF has been an acknowledgement of the risk that is demonstrated by the scientific studies. There is clear evidence of risk, although the magnitude of the risk is*

uncertain, and the magnitude of doing nothing on the health effects cost to society is similarly uncertain. This situation is very similar to our history of dealing with the hazards of smoking decades ago, where the power of the industry to influence governments and even conflicts of interest within the public health community delayed action for more than a generation, with consequent loss of life and enormous extra health care costs to society." (Sage and Carpenter 2009).

2. The population's exposure has increased

Given the range of physiological impacts described in Part 2, the very rapid global deployment of both old and new forms of emerging wireless technologies in the last two decades needs aggressive evaluation from a public health perspective.

In the United States, the deployment of wireless infrastructure (cell tower sites) to support cell phone use has accelerated greatly in the last decades. The Cellular Telephone Institute of America (CTIA) estimated that in 1997 there were only 36,650 cell sites in the US; but increased rapidly to 131,350 in June 2002; 210,350 in June 2007 and 265,561 in June 2012 (Roche, 2012; Cellular Telephone Industry of America (CTIA) 2012). About 220,500 cell sites existed in 2008 (Reardon, 2007; Cellular Telephone Industry of America (CTIA) 2012; Anonymous, May 2005). These wireless facilities for cellular phone voice and data transmission produce RFR over broad areas in communities and are an involuntary and unavoidable source of radiofrequency radiation exposure. Other new RFR exposures that didn't exist before are from WI-FI access points (hotspots) that radiate 24/7 in cafes, stores, libraries, classrooms, on buses and trains, and from personal WI-FI enabled devices (iPads, tablets, PDAs, etc).

Not surprisingly, the use of cell phones has a parallel growth trend. In the late 1980s and early 1990's, only a few percent of the US population were cell phone users. By 2008, eighty-four percent (84%) of the population of the US owned cell phones [16]. CTIA reports that wireless subscriber connections in the US increased from 49 million in June 1997 to 135 million in June 2002 to 243 million in June 2007 to 322 million in June 2012 (Roche, 2012; Cellular Telephone Industry of America (CTIA), June 2012) This represents more than a 100% penetration rate in the US consumer market, up from just a few percent in the early 1990's. The number of wireless subscribers in June 1997 was 18%; in June 2002 it was 47%; in June 2007 it was 81% and in June 2012 it is 101%.

The annualized use of cell phones in the US was estimated to be 2.23 trillion minutes in 2008 and 2.296 trillion minutes in 2010 (CITA, 2012). There are 6 billion users of cell phones world- wide in 2011 up from 2.2 billion in 2008 and many million more users of cordless phones.

The number of US homes with *only* wireless cell phones has risen from 10.5% in 2007 to 31.6% in June of 2012 (Roche, 2012; Cellular Telephone Industry of America (CTIA), June 2012). There are no statistics for June 1997 nor for June 2002, since landline (non-wireless) phone use predominated. The shift to wireless communications, more minutes of use, and reliance on cell and cordless phones rather than corded phones is an extremely revealing measure of new EMF and RFR exposures for both adults and children.

3. Infants, children and childbearing families are highly exposed and vulnerable

With regard to children, the spread of cell towers in communities, often placed on pre-school, church day-care, and school campuses, means that young children may have hundreds of thousands of times higher RF exposures in home and school environments than existed even 20-25 years ago. In addition, the nearly universal switch to cordless and cell phones, and away from corded landline phones, means close and repetitive exposures to both EMF and RFR in the home. Wireless laptops and wireless internet in schools, and home offices and for homework mean even more chronic exposures to RFR, a designated IARC 2B Possible Human Carcinogen (International Agency for Research on Cancer of the World Health Organization, May 2011; Baan, 2011) The great utility of handheld devices as communication aids and sources of information and satisfaction for people on the autism spectrum may come with a concerning underbelly.

Exposures prior to conception or during pregnancy and infancy are also important to consider. These exposures can come from faulty wiring, proximity to power lines, or high-frequency transients from a proximate transformer on a utility pole, or internal sources of pulsed RFR in the home (examples include an electronic baby monitor in the crib, a wireless router in the next room, a DECT phone that pulses high emissions of RFR on a continuous basis 24/7, or conversion to all compact fluorescent bulbs that produce significant 'dirty electricity' for occupants due to low-kilohertz frequency fields on electrical wiring and in ambient space. Sick and vulnerable infants in neonatal intensive care units are heavily exposed from being surrounded by equipment, with negative metabolic and autonomic consequences documented and other likely consequences needing further investigation (Bellieni et al. 2008; Bellieni, Tei, et al. 2012).

Wireless phones and laptops exposures produce extremely low frequency pulses from the battery of the wireless device (Sage, 2007; Sage and Carpenter 2009) and the exposures to pulsed radiofrequency microwave radiation when the wireless device is transmitting or receiving calls and emails.

Especially since EMF/RFR exposures are already classified as IARC 2B Possible Human Carcinogens, we should be actively investigating these sources of damage to DNA that could reasonably result in 'de novo mutations' but also be aware that common environmental exposures from EMF and RFR might play a role in the higher rates of concordance for autism (ASD) among twins and siblings.

Researchers also should be aware that common environmental exposures from EMF and RFR might play a role in the higher rates of autism (ASD) among twins and siblings, not solely because of maternal use of wireless devices during pregnancy and paternal sperm exposure to wireless devices peri-conception; but also because such oxidative damage to DNA can occur at levels introduced into the world of the fetus, and young developing infant and child via baby surveillance monitoring devices in the crib and wireless devices in the home. The deployment of technologies poses risks to human fertility and reproduction capacity, to the fetus, to children and adults (Sage and Carpenter 2009).

4. ASD risk and genomic damage to future generations

Barouki and Grandjean (2012) make a persuasive case that public health interventions are critically needed in early childhood development to prevent adult diseases that appear decades later (Barouki et al. 2012). Although they do not include EMF or RFR but only chemicals, they do say that physiological stressors, which EMF and RFR certainly have been established to be, should be reduced during critical development windows. They say: "*The current pandemic of non-communicable diseases and the increased prevalence of important dysfunctions demand an open interrogation of why current interventions appear insufficient. We now know that disease risk can be induced very early in the life course and that it is modifiable by nutrients and environmental chemical exposures (along with drugs. infections, and other types of stresses)".*

Part II of this chapter documents the detailed scientific basis for considering EMF/RFR exposures to be of significance to the ASDs crisis. Public health interventions are warranted now to protect the genetic heritage of humans, as well as the other stocks of genetic material in wildlife and plants in the face of what appears to be on-going impairment of these genomes. The risk of genomic damage for future generations is sufficiently documented to warrant strong preventative action and new public safety limits that observe EMF/RFR levels shown to cause adverse effects.

5. De-tuning the organism

Genetic mutations may lead to cancer and other diseases in the present and future generations, but the exposures that are capable of creating genotoxic impacts also compromise physiological function Even genotoxicity can have not only specific but also non-specific effects due to inefficiencies, misfolded proteins, and cellular debris, as discussed in the section "Implications of Damage" at the end of the first part of Part II, regarding the rescue of a mouse model of Rett syndrome through enabling a probably generic process of microglial phagocytosis, critical to debris removal, rather than through correcting some specific molecular defect of the synapse (Derecki et al. 2012; Derecki, Cronk, and Kipnis 2012).

In the present setting, where the argument about the pertinence of the cascade of physiological and genotoxic compromises to autism includes the degradative impact on oscillatory synchronization, it is also worth considering that oscillation is a property of living and even physical systems much more generally, and not just of brain oscillatory networks (Strogatz 2003). Under certain circumstances, phase transitions occur and synchronization emerges, often rather quickly rather than gradually – more like a state change than a gradual trend. On the other hand, as mentioned, synchronization can be lost, and there are an enormous number of ways for a system to be de-synchronized, which may relate to the heterogeneity amongst people with ASD that so vexes researchers.

In the setting of autism, a baby gestated or developing as a neonate in a milieu with excessively elevated EMF/RFR exposures is bound to have interference with the normal development processes, including the organization of information and experience in the brain. This baby's environment also often nutritional insufficiencies (processed denatured pesticide-laden food low in antioxidants, minerals and essential fatty acids essential to cellular protection). The baby's gestational period may have been complicated by the mother's own health issues such as conditions like obesity and diabetes (Krakowiak, 2012) which converge on inflammation, oxidative stress and other common forms of physiological dysregulation associated with or even just eating nutrient-depleted, pesticide-laden processed food. The exquisite 'tuning up' of the brain and body as it develops will integrate and respond to the environmental inputs it receives, and is particularly sensitive to environmental miscues (whether chemical like endocrine disruptors, EMF/RFR, or other hostile environmental conditions whether hostile or nurturing). To the extent that the baby is burdened with more disorganized or hostile cues than nurturing and organizing cues, that baby may lose resiliency and become more physiologically vulnerable –perhaps approaching a tipping point into decompensation.

From a systems point of view, the phenomenon of 'autistic regression' may occur after an accumulation of multisystem signaling interference leading to a tipping point of loss of some vital systems synchronization and increase in randomization. EMF/RFR exposures could plausibly contribute both to this vulnerability and to the decompensation/desynchronization process – as could other stressors such as infection, toxicity, acute stress. The vulnerability, then, is the 'allostatic load' – the total burden of stressors pressing toward disorganization. The tipping point may come in a variety of ways but upon investigation one is likely to find that unless it is a severe stressor it is not triggered simply by a single source of stress in an otherwise blissfully healthy child, but rather is the "straw that breaks the camel's back' laid atop a prior accumulation of 'allostatic load.'

C. Conclusions and Recommendations

1. Change our deployment of EMF/RFR

The deployment of RFR from wireless technologies has incredible momentum, and it has made many things easier and many other things possible for the first time. On the other hand this momentum can interfere with setting up the technology in a fashion truly respectful of biological tolerances. Other sections in the Bioinitiative 2012 update will address recommendations and guidelines for increasing the safety profile. This will undoubtedly provoke controversy. The problems will not get settled immediately, and transformation to healthier arrangements will take time.

> *"There is no question that global implementation of the safety standards proposed in the Bioinitiative Report, if implemented abruptly and without careful planning, have the potential to not only be very expensive but also disruptive of life and the economy as we know it. Action must be a balance of risk to cost to benefit. The major risk from maintaining the status quo is an increasing number of cancer cases, especially in young people, as well as neurobehavioral problems at increasing frequencies. The benefits of the status quo are expansion and continued development of communication technologies. But we suspect that the true costs of even existing technologies will only become much more apparent with time. Whether the costs of remedial action are worth the societal benefits is a formula that should reward precautionary behavior.*" (Sage and Carpenter 2009)

2. Encourage precautions right now based on present knowledge

In the meantime many people have already started taking precautionary measures, and more will wish to do so. Physicians and health care people should raise the visibility of EMF/RFR as a plausible environmental factor in clinical evaluations and treatment protocols. Reducing or removing EMF and wireless RFR stressors from the environment is a reasonable precautionary action given the overall weight of evidence.

- Children with existing neurological problems that include cognitive, learning, attention, memory, or behavioral problems should as much as possible be provided with wired (not wireless) learning, living and sleeping environments,
- Special education classrooms should aim for 'no wireless' conditions to reduce avoidable stressors that may impede social, academic and behavioral progress.
- All children should reasonably be protected from the physiological stressor of significantly elevated EMF/RFR (wireless in classrooms, or home environments).
- School districts that are now considering all-wireless learning environments should be strongly cautioned that wired environments are likely to provide better learning and teaching environments, and prevent possible adverse health consequences for both students and faculty in the long-term.

- Monitoring of the impacts of wireless technology in learning and care environments should be performed with sophisticated measurement and data analysis techniques that are cognizant of the non-linear impacts of EMF/RFR and of data techniques most appropriate for discerning these impacts.
- There is sufficient scientific evidence to warrant the selection of wired internet, wired classrooms and wired learning devices, rather than making an expensive and potentially health-harming commitment to wireless devices that may have to be substituted out later, and
- Wired classrooms should reasonably be provided to all students who opt-out of wireless environments.

Undoubtedly risks and the above recommendations will be dismissed by those poised to benefit from the sale of these new systems. Many people also feel that new possibilities have opened up to themselves and the world through wireless technologies. But the public needs to know that these risks exist, that transition to wireless should not be presumed safe, and that it is very much worth the effort to minimize exposures that still provide the benefits of technology in learning, but without the threat of health risk and development impairments to learning and behavior in the classroom.

Broader recommendations also apply, related to reducing the physiological vulnerability to exposures, reduce allostatic load and build physiological resiliency through high quality nutrition, reducing exposure to toxicants and infectious agents, and reducing stress (Herbert and Weintraub 2012), all of which can be implemented safely based upon presently available knowledge.

3. Build an environmentally physiologically centered research program in ASDs as a platform for investigating the EMR/RFR-ASD linkage

This review has been structured around the physiological parallels between ASDs and the impacts of EMF/RFR. What is missing from the autism research agenda is some cross-study of these two bodies of research evidence. To do this we will need both a recognition of the importance of these risks, and a collaborative multi-site research program centered around a “middle-out” physiological approach that can incorporate the the gene-brain-behavior agenda that has dominated ASD research into a broader framework (Herbert 2013). While the middle-out approach is an emerging framework in systems biology that can incorporate complexity and nonlinear, multileveled modeling (Cristofolini et al. 2008; de Graaf et al. 2009; Majumder and Mukherjee 2011; Vinga et al. 2010; Walker and Southgate 2009), the gene-brain-behavior approach has been based on an expectation of linear mapping across the levels on which it focuses, but instead the systems involved appear to be much more complex, and the physiological levels largely left out of this linear approach are critically important to helping people with ASDs because they will help not only with understanding how environment impacts function but also with identifying leverage points.

4. Take the evidence as a call to action

Both EMF and RFR exposures are already classified as IARC 2B Possible Human Carcinogens. The substantial scientific literature on EMF and RFR effects on DNA, on immune and blood-brain barrier disruption, on stress proteins, on circadian rhythms and hormone disregulation, and on cognition, sleep, disruption of neural control and altered brainwave activity all argue for reduction of exposures now, and better coordinated research in these areas.

All relevant environmental conditions should be given weight in defining and implementing prudent, precautionary actions to protect public health, including EMF and RFR. Evidence is sufficient to add EMF/RFR prominently to the list of exposures that can degrade the human genome, and impair normal development, health and quality of our physiology. With the rising numbers people with ASDs and other childhood health and developmental disorders, we cannot afford to ignore this component of risk to our children and vulnerable populations. When the risk factors are largely avoidable or preventable, ignoring clear evidence of large-scale health risks to global populations poses unnecessary and unacceptable risks. Taking this evidence as a call to action will be challenging and disruptive in the short term, but constructive in the longer term as we learn to use EMF/RFR in healthier ways.

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SECTION 22

Precaution in Action – Global Public Health Advice Following BioInitiative 2007

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I. INTRODUCTION

This section highlights some major milestones in documentation of potential health effects of low-intensity electromagnetic fields and radiofrequency radiation, and subsequent national and international actions taken to address the problem. The categories of response are divided into Publications and Health Agency Advisories, Local and National Country Actions, Expert Research Group and Physicians' Advisories and the formal classification by the World Health Organization International Agency for Research on Cancer for RFR as a 2B Possible Human Carcinogen.

II. PUBLICATIONS AND HEALTH AGENCY ADVISORIES (2007 – 2012)

The BioInitiative Report (2007)

The BioInitiative Report (1) is a 650+ page report documenting the evidence for bioeffects and adverse health effects (the science and public health consequences of that body of scientific evidence) from electromagnetic field and radiofrequency (microwave) radiation. It was written by an independent international research group to give an overview of what is known of biological effects that occur at low-intensity EMFs exposures (for both radiofrequency radiation RFR and power-frequency ELF-EMF), and various forms of combined exposures that are now known to be bioactive). The Report examines the research and current standards and finds that these standards are far from adequate to protect public health. The report presents solid science on this issue, and makes recommendations to decision-makers and the public.

The BioInitiative Working Group was composed of scientists, researchers and public health policy professionals. In 2007, the Working Group documented information from over 2000 published scientific studies and reviews reporting bioeffects and adverse health impacts of electromagnetic fields and radiofrequency radiation at exposure levels far below current public safety standards that should be considered in the international debate about the adequacy (or inadequacy) of existing public exposure standards.

Eleven chapters documented key scientific studies and reviews identifying low-intensity effects of electromagnetic fields. Sections 16 and 17 were prepared by public health and policy experts. These sections discuss the standard of evidence which should be applied in public health and environmental planning, how the scientific information should be evaluated in the context of prudent public health policy, and the basis for taking precautionary and preventative actions that are proportionate given this evidence.

European Environment Agency (2007)

European Environmental Agency Executive Director Jacqueline McGlade, PhD provided early support for the BioInitiative Report (2007). The Agency's Head of Communications and Corporate Affairs issued a news release on the publication of the BioInitiative Report, and the EEA contributions to it on September 17, 2007, two weeks after the Report was published on the web. It stated (2):

> *"A new report raising concerns about the effects of electromagnetic fields (EMF) on human health calls for tougher safety standards to regulate radiation from mobile phones, power lines and many other sources of exposure in daily life. The report, 'Bioinitiative: A Rationale for a Biologically-Based Public Exposure Standard for Electromagnetic Fields' was compiled by the BioInitiative Working Group, an international group of scientists, researchers and public health policy professionals. The EEA has contributed to this new report with a chapter drawn from the EEA study 'Late lessons from early warnings: the precautionary principle 1896–2000' published in 2001."*
>
> *"The EEA study reviews the histories of a selection of public and environmental hazards, such as asbestos, benzene and PCBs, from the first scientifically based early warnings about potential harm, to subsequent precautionary and preventive measures. Cases on tobacco smoking and lead in petrol are forthcoming."*
>
> *"Although the EEA does not have specific expertise in EMF, the case studies of public hazards analyzed in the 'Late Lessons from Early Warnings' publication show that harmful exposures can be widespread before there is both 'convincing' evidence of harm from long-term exposures, and biological understanding of how that harm is caused."*
>
> *"There are many examples of the failure to use the precautionary principle in the past, which have resulted in serious and often irreversible damage to health and environments. Appropriate, precautionary and proportionate actions taken now to avoid plausible and potentially serious threats to health from EMF are likely to be seen as prudent and wise from future perspectives. We must remember that precaution is one of the principles of EU environmental policy*."
>
> Professor Jacqueline McGlade, Executive Director, EEA.

In the fall of 2007, the EEA Director responsed to strong media and industry attention to the BioInitiative Report, defending the EEA's position to declare 'early warnings' appropriate with respect to the evidence on mobile phone radiofrequency radiation and possible health hazards. The Director defended EEA recommendations for prudent public health action, based on the scientific evidence presented in the BioInitiative Report. (3)

> *"The BioInitiative report draws attention to some of the emerging evidence of potential harm from the long term effects of non-ionising radiations from electro and magnetic fields (EMF), particularly from the radio frequency (RF) exposures that arise from mobile phone telecommunications."*
>
> *"The Bioinitiative report, however, is only one of several reports reviewing the risks from the*

> *thermal and non-thermal effects of EMF that have been published over recent years."*
>
> *"These include reports from the NIEHS, the EU, the WHO, the UK National Radiological Protection Board and others The EEA's contribution to the BioInitiative report was a chapter on the history and general application of the precautionary principle to a number of well known hazards for which there had been, and in some cases still is, much scientific uncertainty. The chapter summarised the main messages from our report, "Late Lessons from Early Warnings: the Precautionary Principle 1896-2000", (EEA 2001)."*
>
> *"The point of our chapter for the BioInitiative report was to illustrate how past uncertainties had been dealt with so as to provide lessons that may be helpful in dealing with current hazards for which there is both scientific uncertainty and high stakes, both health and economic."*
>
> *"It is because this accumulating evidence on RF is of increasing scientific concern, and because the exposure of the public, particularly vulnerable groups, is widespread and generally rising, that we judged it was timely to draw wider attention to the possibly serious hazards from EMF".*
>
> *"In our judgement, the human and experimental evidence, taken together, is "clear" enough to support using the precautionary principle to justify reducing exposures, where feasible, and to review the evidence for the existing exposure limits, which, as you know, are based on thermal effects only."*
>
> *EEA Director Jacqueline McGlade to Wolfram Konig, Nov 27, 2007*

European Parliament (2007)

In September 26, 2007 Carolyn Lucas, MEP, introduced the topic of the BioInitiative Report recommendations to the European Parliament (4) and asked the European Commission what action the Commission is taking in response to the report, its conclusions and endorsement by the European Environment Agency.

> *"As the Commission will be aware, on 31 August 2007 the international BioInitiative Working Group of renowned scientists and public health policy experts published a report called "A Rationale for a Biologically-Based Public Exposure Standard for Electromagnetic Fields (ELF and RF)".*
>
> *"This report documents evidence that ELFs are a risk factor for both childhood and adult cancers, and sets out how wireless technologies which rely on RF to send emails and voice communications are thousands of times stronger than levels reported to cause sleep disorders, headaches, problems with memory and concentration and other physical symptoms. It notes the unprecedented levels of exposure to ELFs being created by the "explosion of new sources" and raises serious scientific concerns over the health risks posed by long-term and cumulative exposure."*
>
> *"The report concludes that current safety limits regulating the levels of ELF permitted from power lines, mobile phones and other sources are highly inadequate, and that a much more cautious approach should be taken to further deployment of risky technologies."*

> *"The European Environment Agency (EEA) contributed a chapter to the report, concerning the consequences of previous failures to apply the precautionary principle in the face of public and environmental hazards. Following publication of the study the EEA's Executive Director has publicly stressed the importance of precaution where potentially serious future consequences may be involved, and called for actions to reduce exposures to ELF, particularly where vulnerable groups are concerned."*
>
> *"What action is the Commission taking in response to this report, its conclusions and endorsement by the EEA? Does the Commission agree that the balance of evidence points to the need to revise public safety standards regulating radiation levels from sources of day-to-day ELF exposure, as well as policies on the testing and deployment of new telecommunications technologies?"*

European Parliament 2008

The European Parliament issued advice on the Communication from the Commission to the Council, the European Parliament and the European Economic and Social Committee regarding the mid-term review of the European Environment and Health Action Plan 2004-2010 (COM(2007)0314). See Appendix A for full text, but in part, it stated:

> *21. Is greatly concerned at the Bio-Initiative international report (8) concerning electromagnetic fields, which summarises over 1500 studies on that topic and which points in its conclusions to the health risks posed by emissions from mobile-telephony devices such as mobile telephones, UMTS, Wifi, Wimax and Bluetooth, and also DECT landline telephones;*
>
> *22. Notes that the limits on exposure to electromagnetic fields which have been set for the general public are obsolete, since they have not been adjusted in the wake of Council Recommendation 1999/519/EC of 12 July 1999 on the limitation of exposure of the general public to electromagnetic fields (0Hz to 30 GHz) (9, obviously take no account of developments in information and communication technologies, of the recommendations issued by the European Environment Agency or of the stricter emission standards adopted, for example, by Belgium, Italy and Austria, and do not address the issue of vulnerable groups, such as pregnant women, newborn babies and children;*
>
> *23. Calls, consequently, upon the Council to amend its Recommendation 1999/519/EC in order to take into account the Member States' best practices and thus to set stricter exposure limits for all equipment which emits electromagnetic waves in the frequencies between 0.1 MHz and 300 GHz*

Pathophysiology Journal Publication - Special Issue on EMF (2009)

As a direct result of the BioInitiative Report, a special, peer-reviewed issue of Pathophysiology (6) was published in March 2009 and contained most of the BioInitiative content (some chapters updated from 2006 to 2009 published works) including a chapter on public health implications of wireless technologies (7). It also extended the scope of coverage to include RF impacts on the blood-brain barrier, effects of cell towers on wildlife, and reproduction effects in animal studies. It provided assurance of the high scientific quality of the BioInitiative Report analysis and conclusions, and buttressed the need for new EMF safety standards in a respected, peer-reviewed scientific journal.

> *"Bioelectromagnetics, the study of biological effects of electromagnetic fields (EMF), is an interdisciplinary science with a technical literature that is not easily accessible to the non-specialist. To increase access of the public to the technical literature and to the health implications of the scientific findings, the Bioinitiative Report was organized by an international group of scientists and published online at www.bioinitiative.org on August 31, 2007. The report has been widely read, and was cited in September 2008 by the European Parliament when it voted overwhelmingly that the current EMF safety standards were obsolete and needed to be reviewed. "*
>
> *"DNA shows biological effects at the sub-cellular level that occur at very low EMF thresholds and across frequency ranges of the EM spectrum. Interactions with DNA may account for many of the effects of EMF, and they raise the possibility that genetic damage due to EMF can lead to cancer."*
>
> *"The brain is exposed to radiation from mobile phone antennas, and laboratory studies show that the radiation causes leakage of the protective blood–brain barrier, as well as the death of neurons in the brain. Radiation emitted from base stations can affect all who are in the vicinity. Epidemiological studies have shown a relation between exposure to mobile phones, base-stations and the development of brain tumors. Some epidemiological studies have significant flaws in design, and the risk of brain cancer may be greater than reported in the published results."*
>
> *"In addition to the risk of brain cancer, EMF in the environment may contribute to diseases like Alzheimer's dementia and breast cancer in humans, as well as reproductive and developmental effects in animals in the wild. EMF affects the biochemical pathways and immunological mechanisms that link the different organ systems in our bodies and those of animals. The human body can act as an antenna for RF signals, and a small percentage of the population appears to be so sensitive to EMF that it interferes with their daily lives. In addition to the growing presence of EMF signals in the environment, the complexity of the signals may be important in altering biological responses. These are among the many factors that must be considered in approaching EMF safety issues."*
>
> Preface, Pathophysiology, Guest Editor Martin Blank, PhD

Media coverage of the Pathophysiology Journal in 2009 highlighted the everyday problems of EMF and wireless exposures in society. For the typical person on the street, the message of the BioInitiative Report and its subsequent contributions to a scientific journal were broken down into examples more familiar to them (8).

> *"Public health concerns and scientific evidence for risks from cell phones and other wireless devices is published today in the journal Pathophysiology. International researchers have urged quick precautionary action to address a possible epidemic of brain tumors and many other health risks. Over four billion people around the world now use cell phones. They are rapidly eliminating the use of traditional land-line phones throughout the world. Health researchers from six countries give findings in fifteen (15) chapters covering health risks to humans and wildlife from electromagnetic fields and radiofrequency radiation."*
>
> *"The global rollout of wireless technologies and devices like cell phones, cordless phones, cell towers (masts) and many other sources greatly increases our EMF exposure in daily life. The enormous popularity of new communication devices that allow email, texting, and access to the Internet from any city street has placed the issue squarely before government agencies like the FDA and the FCC, and also parents and school administrators. Parents must decide whether possible health risks to their children outweigh the convenience of keeping track of them. School officials and teachers care because of disruption and distraction in the classroom from cell phone use. National safety officials in the US face public criticism about highway collisions and road deaths from cell phone use while driving. Federal railway officials are*

still coping with the problem of illicit texting by US railroad personnel that lead to the catastrophic collision of two trains in Chatsworth, California in 2008 killing 24 and injuring 135 more."

Reba Goodman, PhD (Columbia University) commented: "*cells in the body react to EMFs as potentially harmful, just like to other environmental toxins including heavy metals and toxic chemicals. The DNA in living cells recognizes electromagnetic fields at very low levels of exposure, and produces a biochemical stress response."*

David O. Carpenter, MD, Co-Editor of the BioInitiative Report and Director of the University of Albany, Institute of Health and the Environment concluded: "*the existing FCC and international limits do not do enough to protect people, especially children, from daily exposures to electromagnetic fields and radiofrequency radiation. The existing safety limits did not anticipate these new kinds of technologies affecting the health of people living with and using wireless devices on a daily basis. These effects are now widely reported to occur at exposure levels significantly below most current national and international limits."*

Brain tumor specialist Dr. Lennart Hardell, MD, PhD works as both an oncologist and a researcher at Orebro University Hospital in Sweden. He is an expert on cell phones and brain tumors.

"The evidence for risks from prolonged cell phone and cordless phone use is quite strong. For people who have used these devices for 10 years or longer, and when they are used mainly on one side of the head, the risk of malignant brain tumor is doubled for adults and is even higher for persons with first use before the age of 20 years."

Swedish researcher Olle Johansson, PhD (Karolinska Institute) said: "*most worrisome to me are the constant and unavoidable EMF exposures (from cell and DECT phones, power lines, new wireless technologies like WIMAX and WI-FI, etc.) everywhere in our daily life that may affect the overall health of this and coming generations. I worry especially about the impacts on the immune system, our only real line of defense against disease."*

Wildlife biologist Alfonso Balmoro, PhD of Valladolid, Spain voiced his concern that:*"electromagnetic radiation is a form of environmental pollution which may hurt wildlife. Phone masts located in their living areas are irradiating continuously some species that could suffer long-term effects, like reduction of their natural defenses, deterioration of their health, problems in reproduction and reduction of their useful territory through habitat deterioration. Therefore microwave and radiofrequency pollution constitutes a potential cause for the decline of animal populations and deterioration of health of plants living near phone masts."*

Co-Editor of the BioInitiative Report Cindy Sage observed: *"Prolonged exposure to radiofrequency and microwave radiation from cell phones, cordless phones, cell towers, WI-FI and other wireless technologies has been linked to interference with short-term memory and concentration, sleep disruption, headache and dizziness, fatigue, immune disruption, skin rashes and changes in cardiac function."*

"*these effects can happen with even very small levels of exposure if they occur on a daily basis. Cell phone use is likely to be more harmful in children whose brain and nervous system development can last into late adolescence*"

"The public health implications of billions of people who are exposed makes this a matter of critical concern to policy-makers around the world."

The European Environment Agency Director's Statement (2009)

Two years following the publication of the BioInitiative Report, and just months after publication of the special issue of Pathophysiology on EMF, the EEA updated its comments on potential health risks of EMF and concern over the adequacy of public safety limits for emerging wireless technologies. The EEA issued a Statement on Mobile Phones for the September, 2009 conference 'Cell Phones and Health: Science and Public Policy Questinos, Washington DC. In part, the comments read (9):

> *"This event and the related Senate Hearings yesterday, have been, in part, stimulated by the BioIntiative Report, (2007), which helped increase public awareness of the potential hazards of electromagnetic fields, not least from mobile phones. The European Parliament responded to this debate with its resolution earlier this year which, among other things, called for lowering exposure to electromagnetic fields and for new exposure limits that would better protect the public. We fully share these recommendations."*

The EEA provides data, information and knowledge on the environment, including its impacts on public health, to EU institutions (the European Parliament, European Commission, and European Council of Ministers), to the 32 Member Countries of the EEA, and to the general public.

> *"The intention of the EEA to promote the use of mobile telephony in this way increases its responsibility to provide information that can help ensure the safety of the public when using mobile phones, especially vulnerable groups such as children, the elderly, and the immuno- compromised. This is the reason why the EEA issued an early warning about the potential hazards of EMF on 17 September 2007."*
>
> *"In this we drew attention to the BioInitiative report and to the other main references relevant to this debate (from the EU, the WHO, and the UK National Radiological Protection Board) which, taken together, provided the basis for our early warning on EMF."*
>
> *"Specifically, we noted that there are many examples of the failure to use the precautionary principle in the past, which have resulted in serious and often irreversible damage to health and environments. Appropriate, precautionary and proportionate actions taken now to avoid plausible and potentially serious threats to health from EMF are likely to be seen as prudent and wise from future perspectives".*
>
> *"This is the reason why the EEA issued an early warning about the potential hazards of EMF on 17 September 2007."*

EEA Recommendations based on current evidence (2009)
The evidence is now strong enough, using the precautionary principle, to justify the following steps:

1. *For governments, the mobile phone industry, and the public to take all reasonable measures to reduce exposures to EMF, especially to radio frequencies from mobile phones, and particularly the exposures to children and young adults who seem to be most at risk from head tumours. Such measures would include stopping the use of a mobile phone by placing it next to the brain. This can be achieved by the use of texting; hands free sets; and by the use of phones of an improved design which could generate less radiation and make it convenient to use hands free sets.*
2. *To reconsider the scientific basis for the present EMF exposure standards which have serious limitations such as reliance on the contested thermal effects paradigm; and simplistic assumptions about the complexities of radio frequency exposures.*
3. *To provide effective labeling and warnings about potential risks for users of mobile phones.*
4. *To generate the funds needed to finance and organise the urgently needed research into the health effects of phones and associated masts. Such funds could include grants from industry and possibly a small levy on the purchase and or use of mobile phones. This idea of a research levy is*

> *a practice that we think the US pioneered in the rubber industry with a research levy on rubber industry activities in the 1970s when lung and stomach cancer was an emerging problem for that industry. The research funds would be used by independent bodies.*

European Parliament 2009

On April 2, 2009, the European Parliament adopted the "European Parliament resolution of 2 April 2009 on health concerns associated with electromagnetic fields (2008/2211(INI))" (10). The Document was based on the "Report on health concerns associated with electromagnetic fields", Rapporteur: Frederique Ries (11) Committee on the Environment, Public Health and Food Safety. See Appendix B for full text. It part, the resolution says:

> *H. (W)hereas, however, there are some points that appear to be the subject of general agreement, in particular the idea that reactions to microwave exposure vary from one person to another, the need, as a matter of priority, to conduct exposure tests under actual conditions in order to assess the non-thermal effects associated with radio-frequency (RF) fields, and the fact that children exposed to EMFs are especially vulnerable (9) ,*
>
> *1. Urges the Commission to review the scientific basis and adequacy of the EMF limits as laid down in Recommendation 1999/519/EC and report to the Parliament; calls for the review to be undertaken by the Scientific Committee on Emerging and Newly Identified Health Risks;*
>
> *2. Calls for particular consideration of biological effects when assessing the potential health impact of electromagnetic radiation, especially given that some studies have found the most harmful effects at lowest levels; calls for active research to address potential health problems by developing solutions that negate or reduce the pulsating and amplitude modulation of the frequencies used for transmission;*
>
> *5. Invites the Member States and local and regional authorities to create a one-stop shop for authorisation to install antennas and repeaters, and to include among their urban development plans a regional antenna plan*
>
> *6. Urges the authorities responsible for authorising the siting of mobile telephony antennas to reach agreement, jointly with the operators in that sector, on the sharing of infrastructure, in order to reduce the volume thereof and the exposure of the public to EMFs;*
>
> *15. Draws attention in this context to the appeal for caution from the coordinator of the Interphone study, Elisabeth Cardis, who, in the light of existing knowledge, recommends, as far as children are concerned, that mobile phones should not be used beyond reasonable limits and that landlines should be preferred;*
>
> *21. Calls on the Commission, in recognition of the public concern in many Member States, to work with all relevant stakeholders, such as national experts, non-governmental organisations and industrial sectors, to improve the availability of, and access to, up-to-date information understandable to non-specialists on wireless technology and protection standards;*
>
> *24. Proposes that the EU's indoor air quality policy should encompass the study of "wireless" domestic appliances, which, like Wi-Fi for Internet access and digital enhanced cordless telecommunications (DECT) telephones, have been widely adopted in recent years in public places and in the home, with the result that citizens are being continuously exposed to microwave emissions;*

29. Instructs its President to forward this resolution to the Council, the Commission, the

This revision essentially neutralized the chance for an independent and unbiased review of health effects and assessment of the adequacy of the ICNIRP/FCC thermally-based public health standards by designating the SCENIHR Committee to be the arbiter. The SCENIHR Committee ignored the non-thermal science and public health issues on EMF in past reviews. Appointing SCENIHR to provide the 'official' report to Parliament on health effects of EMF essentially guaranteed the outcome would be ineffectual for precautionary action, that the standard of evidence for judging would be "causal" evidence; and a public health standard for judging the evidence would not prevail. Reaching the very high bar of establishing 'causal evidence of risk' is not in line with precautionary, prudent public health decision-making. It will delay necessary actions for avoidance long past the 'early warning' stage when such actions may reasonably prevent substantial health harm.

However many points adopted in the resolution are in favour of public health and must not be dismissed.

Seletun Statement 2009

In November, 2009, a scientific panel met in Seletun, Norway, for three days of intensive discussion on existing scientific evidence and public health implications of the unprecedented global exposures to artificial electromagnetic fields (EMF). The Scientific Panel recognized that the body of evidence on EMF requires a new approach to protection of public health; the growth and development of the fetus, and of children; and argues for strong preventative actions. The study concluded that "new, biologically-based public exposure standards are urgently needed to protect public health worldwide" (12).

The Seletun Statement was published in 2010 in the journal Reviews on Environmental Health. It was titled *Scientific panel on electromagnetic field health risks: Consensus points, recommendations, and rationales*. Scientific Meeting: Seletun, Norway, November 17-21, 2009. (12).

Specific Recommendations from the Seletun Scientific Panel are:

"Extremely Low Frequency (Fields from Electrical Power)

- *Based on the available evidence, the Seletun Scientific Panel recommends a 0.1 uT (1 mG) exposure limit for all new installations based on findings of risk for leukemia, brain tumours,* *Alzheimer's, ALS, sperm damage and DNA strand breaks. This exposure limit does not include a safety margin;*

- *For all newly installed, or newly upgraded electrical power distribution, the Panel recommends a 0.1 uT (1 mG) set-back distance, from residences, hospitals, schools, parks, and playgrounds schools (and similar locations occupied by children) [A 0.1 uT (1 mG) time-weighted average (TWA) using peak loading for transmission lines to ensure that average is about half of this for typical exposures; or equivalent for long-term exposure in interior EMF environments (wiring, trans-formers, appliances, others).];*
- *For all newly constructed residences, offices, schools (and other facilities with children), and hospitals there shall be a 0.1 uT (1 mG) max. 24 hour average exposure limit;*
- *For all new equipment (e.g. transformers, motors, electronic products), where practical, the Panel recommends a 0.1 uT (1 mG) max. 24 hour average exposure limit. Where not practical (e.g. large power transformers), there should be a fence, or boundary marker, with clearly written warning labels that states that within the boundary area the 0.1 uT (1 mG) maximum, 24 hour average exposure limit is exceeded;*
- *The Panel recommends all countries should adopt electrical code requirements to disallow conduction of high-frequency voltage transients back into electrical wiring systems;*
- *All new electronic devices including compact fluorescent lamps (CFLs) should be constructed with filters to block high-frequency voltage transients from being conducted back onto electrical wiring systems;*
- *The Panel recommends electric field reductions from electrical wiring in buildings based on evidence of increased cancer risk from prolonged or repetitive electric field exposure. The United States National Electrical Code (NEC) and other govern-mental codes relating to building design and construction should be revised so that all new electrical wiring is enclosed in a grounded metal shield;*
- *The United States NEC and other govern-mental codes that disallow net current on electrical wiring should be better enforced, and ground fault interrupters (GFIs) should be installed on all electrical circuits in order to reduce net current.*

Radiofrequency/Microwave Radiation Exposure Limit Recommendations

- *Present guidelines, such as IEEE, FCC, and ICNIRP, are not adequate to protect humans from harmful effects of chronic EMF exposure. The existing scientific knowledge is, however, not sufficient at this stage to formulate final and definite science-based guidelines for all these fields and conditions, particularly for such chronic exposure as well as contributions of the different parameters of the fields, e.g. frequency, modulation, intensity, and window effects. The values suggested below are, thus, provisional and may be altered in the future.*
- *For whole-body (in vivo experiments) or cell culture-based exposure, the Seletun Scientific Panel finds sufficient evidence to establish a scientific benchmark for adverse health effect at 0.0166 W/kg based on at least 32 scientific studies reporting low-intensity effects (defined as studies reporting effects at exposures of 0.1 W/kg or lower) /8-39/.*
- *The Panel recommends a provisional whole-body limit of 0.00033 W/kg by incorporation of an additional 50-fold safety margin applied to the scientific benchmark of 0.0166 W/kg. This is consistent with both ICNIRP and IEEE/FCC safety factors. An additional 10-fold reduction is applied to take prolonged exposure into account (because 29 of the 32 studies are acute exposure only), giving a final whole-body limit of 0.000033 W/kg (33 µW/kg). No further safety margin or provision for sensitive populations is incorporated. This may need to be lowered in the future.*

- *Based on power density measurements, the Seletun Scientific Panel finds sufficient evidence for a whole-body scientific bench-mark for adverse health effect exists down to 85 mW/m^2 (0.0085 mW/cm^2 or 8.5 μW/cm^2) based on at least 17 scientific studies reporting low-intensity effects on humans. Taking more recent human studies conducted near base stations, or at base-station RF levels, Kundi and Hutter /57/ report that the levels must exceed 0.5-1.0 mW/m^2 (0.05 to 0.1 uW/cm^2) for effects to be seen; /40-57/.*

- *The Panel recommends a provisional whole-body (far-field) limit of 1.7 mW/m^2 (also = 0.00017 mW/cm^2 = 0.17 μW/cm^2) by incorporation of an additional 50-fold safety margin applied to the scientific benchmark of 85 mW/m^2. This is consistent with both ICNIRP and IEEE/FCC safety factors. This may need to be lowered in the future.*

- *It can be argued that a further 10-fold reduction is not justified since 13 of the 17 studies are already testing for long-term RF exposure. However, considering that the latest human population studies as reported by Kundi & Hutter (2009) do not show effects below 0.5-1.0 mW/m2, it can also then be argued that an additional 10-fold reduction on precautionary grounds is justified. If another 10-fold reduction is applied, the recommended level would then be 0.17 mW/m^2 (also 0.000017 mW/cm^2 = 0.017 μW/cm^2);*

- *The Seletun Scientific Panel recommends these numeric limits to governments and health agencies for adoption in place of ICNIRP, IEEE/FCC and other outdated public safety guidelines and limits in use around the world. This approach is based on traditional public health principles that support taking actions to protect public health when sufficient evidence is present. Sufficient scientific evidence and public health concern exist today based on increased risk for cancer, adverse fertility and reproductive outcomes, immune disruption, neurological diseases, increased risk of road collisions and injury-producing events, and impairment of cognition, behaviour, performance, mood status, and disruption of sleep;*

- *Numeric limits recommended here do not yet take into account sensitive populations (EHS, immune-compromised, the fetus, developing children, the elderly, people on medications, etc). Another safety margin is, thus, likely justified further below the numeric limits for EMF exposure recommended here;*

- *The Scientific Panel acknowledges that numeric limits derived here for new biologically-based public exposure standards are still a billion times higher than natural EMF levels at which all life evolved.*

Specific Recommendations for mobile (cell) and cordless phone use

- *The Seletun Scientific Panel recommends that users keep mobile (cell) phones away from head and body;*

- *The Seletun Scientific Panel recommends that users keep mobile (cell) phones and PDAs* switched off if worn or carried in a pocket or holster, or on a belt near the body. *PDA is generic for any type of Personal Digital Assistant or hand-held computer device;*

- *The Panel strongly recommends against the use of mobile (cell) and cordless phones and PDAs by children of any age;*

- *The Panel strongly recommends against the use of mobile (cell) and cordless phones and PDAs by pregnant women;*

- *The Panel recommends that use of mobile (cell) and cordless phones and PDAs be curtailed near children or pregnant women, in keeping with preventative and precautionary strategies. The most vulnerable members of society should have access to public places without fear of harm to health;*

- *Public access to public places and public transportation should be available without undue risk of*

EMF exposure, particularly in enclosed spaces (trains, airplanes, buses, cars, etc) where the exposure is likely to be involuntary;

- *The Panel recommends wired internet access in schools, and strongly recommends that schools do not install wireless internet connections that create pervasive and prolonged EMF exposures for children;*

- *The Panel recommends preservation of existing land-line connections and public telephone networks;*

- *The Panel recommends against the use of cordless phones (DECT phones) and other wireless devices, toys and baby monitors, wireless internet, wireless security systems, and wireless power transmitters in SmartGrid-type connections that may produce unnecessary and potentially harmful EMF exposures;*

- *The Panel recognizes that wired internet access (cable modem, wired Ethernet connections, etc) is available as a substitute;*

- *The Panel recommends use of wired headsets, preferably with hollow-tube segments;*

- *The Panel recommends avoidance of wireless (Bluetooth-type) headsets in general;*

- *The Panel encourages the removal of speakers from headsets on wireless phones and PDAs;*

- *The Panel encourages ‗auto-off switches' for mobiles (cells) and PDAs that automatically turn off the device when placed in a holster;*

- *The Panel strongly discourages the technology that allows one mobile (cell) phone to act as a repeater for other phones within the general area. This can increase exposures to EMF that are unknown to the person whose phone is ―piggy-backed upon without their knowledge or permission;*

- *The Panel recommends the use of telephone lines (land-lines) or fiber optic cables for SmartGrid type energy conservation infra-structure. Utilities should choose options that do not create new, community-wide exposures from wireless components of SmartGrid-type projects. Future health risks from prolonged or repetitive wireless exposures of SmartGrid-type systems may be avoided by using telephone lines or fiber-optic cable. The Panel endorses energy conservation but not at the risk of exposing hundreds of millions of families in their homes to a new, involuntary source of wireless radiofrequency radiation."*

Ten Key points had been identified:

- *"The global populations are insufficiently protected, thus currently at risk;*

- *Sensitive Populations are extra vulnerable;*

- *Government actions are urgently warranted now, based on evidence of serious disruption to biological systems;*

- *The Burden of Proof for the safety of radiation-emitting technologies should fall on Producers and Providers, not Consumers;*

- *EMF Exposures should be reduced in advance of complete understanding of mechanisms of action;*

- *The current operative measure of Radiation Risk - the specific absorption rate (SAR) - is inadequate, and misguides on safety and health risks;*

- *An international Disease Registry is needed to track Time Trends of the incidence of Illnesses to correlate the illnesses with exposures;*

- *Pre-market health testing and safety demonstration is needed for all radiation-emitting technologies;*
- *Parity is needed for occupational exposure standards, compared to those for the general public;*
- *Persons with Electrohypersensitivity need the classification Functionally Impaired.*
- *The scientists recommend specific exposure limits for different frequency fields, including microwaves, used in wireless communications, and ELF electric fields and magnetic fields."*

Collegium Ramazzini Publication (2010)

The 400 page review of non-thermal EMF effects by the Ramazzini Institute, and sponsored by the International Commission for Electromagnetic Safety, and the National Institute for the Study and Control of Cancer and Environmental Diseases 'Bernardino Ramazzini' in 2010 provided a substantial evidence foundation for the relationship between low-intensity EMF (ELF-EMF and RFR) exposure and potential health risks (13). Taken as a whole, the two-volume report provides a compelling scientific basis on which to take precautionary, prudent public health actions. The EEA relied heavily on the Collegium Ramazzini publication to buttress their Statement on Mobile Phones, when addressing the Council of Europe the following year.

European Environment Agency (2011)

Dr. Jacqueline McGlade, Executive Director of the European Environment Agency provided key guidance to the Council of Europe in her *Statement on Mobile Phones and the Potential Head Cancer Risk for EMF* to the Council of Europe, Paris, February 25th 2011 (14). It read:

> *"The European Parliament[1] has responded to this public concern with a resolution on EMF in 2009 which, among other things, called for lowering exposure to electromagnetic fields and for lower exposure limits that would better protect the public from health hazards. We share these recommendations."*

[1] European Parliament resolution of 2 April 2009 on health concerns associated with electromagnetic fields (2008/2211(INI))

Further, she urged the Council of Europe take interim actions to protect public health, particularly for children, with the following:

> *"The EU Commission and the EEA sees the precautionary principle as central to public policymaking where there is scientific uncertainty and high health, environmental and economic costs in acting, or not acting, when faced with conflicting evidence of potentially serious harm."*
>
> *"This is precisely the situation that characterises EMF at this point in its history. Waiting for high levels of proof before taking action to prevent well known risks can lead to very high health and economic costs, as we have seen with asbestos, leaded petrol and smoking."*

Council of Europe 2011

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[1] European Parliament resolution of 2 April 2009 on health concerns associated with electromagnetic fields (2008/2211(INI))

On May 27, 2011 the Standing Committee, acting on behalf of the Parliamentary Assembly of the Council of Europe (PACE), adopted the Resolution 1815 (2011) “The potential dangers of electromagnetic fields and their effect on the environment” (15) based on the Doc. 12608, report of the Committee on the Environment, Agriculture and Local and Regional Affairs, rapporteur: Mr Huss (16). The Parliamentary Assembly of the Council of Europe come from the national parliaments of the Organization’s 47 member states and speak for the 800 million Europeans who elected them. The texts adopted by PACE – recommendations, resolutions and opinions – serve as guidelines for the Committee of Ministers, national governments, parliaments and political parties (17).

Recommendations given by the PACE Resolution 1815:

> *“8. In light of the above considerations, the Assembly recommends that the member states of the Council of Europe:*
>
> ***8.1. in general terms:***
>
> *8.1.1. take all reasonable measures to reduce exposure to electromagnetic fields, especially to radio frequencies from mobile phones, and particularly the exposure to children and young people who seem to be most at risk from head tumours;*
>
> *8.1.2. reconsider the scientific basis for the present standards on exposure to electromagnetic fields set by the International Commission on Non-Ionising Radiation Protection, which have serious limitations, and apply ALARA principles, covering both thermal effects and the athermic or biological effects of electromagnetic emissions or radiation;*
>
> *8.1.3. put in place information and awareness-raising campaigns on the risks of potentially harmful long-term biological effects on the environment and on human health, especially targeting children, teenagers and young people of reproductive age;*
>
> *8.1.4. pay particular attention to “electrosensitive” people who suffer from a syndrome of intolerance to electromagnetic fields and introduce special measures to protect them, including the creation of wave-free areas not covered by the wireless network;*
>
> *8.1.5. in order to reduce costs, save energy, and protect the environment and human health, step up research on new types of antenna, mobile phone and DECT-type device, and encourage research to develop telecommunication based on other technologies which are just as efficient but whose effects are less negative on the environment and health;*
>
> ***8.2. concerning the private use of mobile phones, DECT wireless phones, WiFi, WLAN and WIMAX for computers and other wireless devices such as baby monitors:***
>
> *8.2.1. set preventive thresholds for levels of long-term exposure to microwaves in all indoor areas, in accordance with the precautionary principle, not exceeding 0.6 volts per metre, and in the medium term to reduce it to 0.2 volts per metre;*
>
> *8.2.2. undertake appropriate risk-assessment procedures for all new types of device prior to licensing;*

8.2.3. introduce clear labelling indicating the presence of microwaves or electromagnetic fields, the transmitting power or the specific absorption rate (SAR) of the device and any health risks connected with its use;

8.2.4. raise awareness on potential health risks of DECT wireless telephones, baby monitors and other domestic appliances which emit continuous pulse waves, if all electrical equipment is left permanently on standby, and recommend the use of wired, fixed telephones at home or, failing that, models which do not permanently emit pulse waves;

8.3. concerning the protection of children:

8.3.1. develop within different ministries (education, environment and health) targeted information campaigns aimed at teachers, parents and children to alert them to the specific risks of early, ill-considered and prolonged use of mobiles and other devices emitting microwaves;

8.3.2. for children in general, and particularly in schools and classrooms, give preference to wired Internet connections, and strictly regulate the use of mobile phones by schoolchildren on school premises;

8.4. concerning the planning of electric power lines and relay antenna base stations:

8.4.1. introduce town planning measures to keep high-voltage power lines and other electric installations at a safe distance from dwellings;

8.4.2. apply strict safety standards for the health impact of electrical systems in new dwellings;

8.4.3. reduce threshold values for relay antennae in accordance with the ALARA principle and install systems for comprehensive and continuous monitoring of all antennae;

8.4.4. determine the sites of any new GSM, UMTS, WiFi or WIMAX antennae not solely according to the operators' interests but in consultation with local and regional government authorities, local residents and associations of concerned citizens;

8.5. concerning risk assessment and precautions:

8.5.1. make risk assessment more prevention oriented;

8.5.2. improve risk-assessment standards and quality by creating a standard risk scale, making the indication of the risk level mandatory, commissioning several risk hypotheses to be studied and considering compatibility with real-life conditions;

8.5.3. pay heed to and protect "early warning" scientists;

8.5.4. formulate a human-rights-oriented definition of the precautionary and ALARA principles;

8.5.5. increase public funding of independent research, in particular through grants from industry and taxation of products that are the subject of public research studies to evaluate health risks;

8.5.6. create independent commissions for the allocation of public funds;

8.5.7. make the transparency of lobby groups mandatory;

> *8.5.8. promote pluralist and contradictory debates between all stakeholders, including civil society (Århus Convention)."*

European Environment Agency 2011

In October 12 2011, the European Environment Agency (EEA), an agency of the European Union. based in Copenhagen, Denmark, recommends again to take a precautionary approach to policy making in the EMF area (18). The Agency notes:

"The precautionary principle.

> *Because the evidence on mobile phones and cancer presents a mixed picture, the EEA recommends using the precautionary principle (PP), as recommended in the EU Treaty, to better manage the risk. There is no clear legal definition of the PP so the EEA has produced a working definition:*
>
> *The precautionary principle provides justification for public policy actions in situations of scientific complexity, uncertainty and ignorance, where there may be a need to avoid, or reduce, potentially serious or irreversible threats to health and the environment, using an appropriate strength of scientific evidence, and taking into account the pros and cons of action and inaction.*
>
> *The PP requires us to weigh evidence in a different way. This is not new - societies are used to using different strengths of evidence for different reasons, based on the costs of being wrong. For example, criminals must be found guilty 'beyond all reasonable doubt' before they are convicted; injured people in compensation cases need only show a balance of evidence in order to win compensation for negligence; while doctors only need slight evidence of a serious illness to prescribe treatment. Such precautionary approaches are justified where it is not yet possible to establish causality beyond reasonable doubt.*
>
> *Implications for policy makers and the mobile phone industry.*
>
> *Citizens could be better informed about the risks of mobile phone use, as recommended by the EEA in September 2007. There is sufficient evidence of risk to advise people, especially children, not to place the handset against their heads: text messaging, or hands-free kits lead to about ten times lower radiation levels, on average, than when the phone is pressed to the head.*
>
> *Governments may also wish to label mobile handsets as a 'possible carcinogen', in line with the IARC decision. In addition, more independent research is needed. The cost of these measures is very low, but the potential costs of inaction may be very high."*

US Government Accountability Office (2012)

The US Government Accountability Office published a report in 2012 urging the US Federal Communications Commission to revisit the outdated safety standards for the exposures from wireless devices. (19)

The rapid adoption of mobile phones has occurred amidst controversy over whether the technology poses a risk to human health as a result of long-term exposure to RF energy from mobile phone use.

FCC and FDA share regulatory responsibilities for mobile phones. GAO was asked to examine several issues related to mobile phone health effects and regulation. Specifically, this report addresses:

1. *(1) what is known about the health effects of RF energy from mobile phones and what are current research activities,*
2. *(2) how FCC set the RF energy exposure limit for mobile phones, and*
3. *(3) federal agency and industry actions to inform the public about health issues related to mobile phones, among other things.*
4. *GAO reviewed scientific research; interviewed experts in fields such as public health and engineering, officials from federal agencies, and representatives of academic institutions, consumer groups, and the mobile phone industry; reviewed mobile phone testing and certification regulations and guidance; and reviewed relevant federal agency websites and mobile phone user manuals.*

The Report noted that the FCC's RF energy exposure limit may not reflect the latest research. Redundant and overlapping jurisdiction over the setting of public safety limits is highlighted where the GAO Report notes:

> *"FCC told GAO that it relies on the guidance of federal health and safety agencies when determining the RF energy exposure limit, and to date, none of these agencies have advised FCC to change the limit. However, FCC has not formally asked these agencies for a reassessment. By not formally reassessing it's current limit, FCC cannot ensure it is using a limit that reflects the latest research on RF energy exposure. FCC has also not reassessed it's testing requirements to ensure that they identify the maximum RF energy exposure a user could experience. Some consumers may use mobile phones against the body, which FCC does not currently test, and could result in RF energy exposure higher than the FCC limit." (US GAO, 2012)*

The GAO Report recommends to the FCC that it formally reassess, and, if appropriate, change it's current RF energy exposure limit and mobile phone testing requirements related to likely usage configurations, particularly when phones are held against the body. FCC noted that a draft document that is now under consideration by the FCC has the potential to address GAO's recommendations. (US GAO, 2012)

European Environment Agency: Late Lessons II - Mobile Phone Chapter (2012)

The European Environment Agency (EEA) has Late Lessons from Early Warnings: Science, Precaution, Innovation (20). It includes a new chapter on Mobile Phone Use and Brain Tumor Risk (Hardell et al., 2012 (21). It addresses the early 'lessons' learned about carcinogenicity of EMF hazards from power lines and visual display units or VDUs. ELF-EMF was classified in 2001 by IARC as a 2B Possible Human Carcinogen. It provides a chronology of the publication of studies, including the Final Interphone Report, the combined Hardell et al. papers (1999-2011) on brain tumor risks, and finally the classification in 2011 by IARC of radiofrequency radiation also to be a Group 2B Possible Human Carcinogen. The paper includes a section on risks to children. It shows that for children who start using a mobile phone in their early teenage years, by the time these children are in the 20-29 age group, they have a 500%+ increased risk of glioma and a 600%+ increased risk of acoustic neuroma when they are young adults. The risks for adults (ipsilateral, 10+ years of mobile phone use are roughly 200% or doubled.

III. EXPERT RESEARCH GROUP AND PHYSICIANS' ADVISORIES (2007 – 2012)

American Academy of Environmental Medicine Statement

In a landmark statement adopted early 2012, the American Academy of Medicine (AAEM) signaled it's opposition to the California Public Utilities Commission proposal to install wireless utility meters in California that create new sources of elevated radiofrequency radiation wherever buildings have electrical meters (22 and Appendix C). The letter stated:

> *"The American Academy of Environmental Medicine opposes the installation of wireless 'smart meters' in homes and schools based on a scientific assessment of the current medical literature (references available on request). Chronic exposure to wireless radiofrequency radiation is a preventable environmental hazard that is sufficiently well-documented to warrant immediate preventative public health action."*

The American Academy of Environmental Medicine was founded in 1965, and is an international association of physicians and other professionals interested in the clinical aspects of humans and their environment. The Academy is interested in expanding the knowledge of interactions between human individuals and their environment, as these may be demonstrated to be reflected in their total health. The AAEM provides research and education in the recognition, treatment and prevention of illnesses induced by exposures to biological and chemical agents encountered in air, food and water. This represents the first national physician's group to look in-depth at wireless health risks; and to advise the public and decision-makers about preventative public health actions that are necessary. The AAEM based its opinion in part on the established scientific evidence, and on the recent classification by the WHO International Agency for Research on Cancer (IARC) that radiofrequency radiation, like ELF-EMF is a Group 2B Possible Human Carcinogen. The rationale for widespread public exposure to a new source of radiofrequency radiation in every home and classroom, after being designated a Possible Human Carcinogen, is clearly unacceptable from a medical and public health standpoint. The full text of the letter is Appendix A.

International Doctors′ Appeal (2012)

In 2002 more than 1000 physicians signed the “Freiburg Appeal” (23). It was translated into many languages. As many as 36,000 people from all over the world support its warning about the dangers of wireless communication. Ten years later, in October 2012 the ‘International Doctors′ Appeal 2012’ was published (24).

“As physicians and scientists, we hereby call on our colleagues and the wider global community to support us with their signature in our fight for the protection of life. However, we also appeal to the politicians to ensure that the people are protected by the following precautionary measures, which also include fundamental human rights:

- *Protect the inviolability of the home by minimizing radio-frequency exposure levels, which penetrate through the walls of one's own home.*
- *Considerably lower radio-frequency radiation exposures as well as exposure limits to a level that reliably protects humans and nature from adverse biological effects of electromagnetic fields.*
- *Convert devices/transmitters that transmit continuously (e.g. cordless phones, wireless Internetaccess (Wi-Fi), and wireless meters) to technologies that only emit radio-frequency radiation on demand when being used.*
- *Children and adolescents need special protection: Children below the age of 8 should not use cell phones and cordless phones; children and adolescents between the ages 8 and 16 should not use cell phones or only use them in the case of an emergency.*
- *Attach clearly visible warning labels and safety guidelines for lowering the radiation exposure on cell phones and other wireless devices, including instruction manuals. An important reminder: Try not to carry a cell phone right next to your body when it is turned on.*
- *Identify and clearly mark protected zones for electrohypersensitive people; establish public areas without wireless access or coverage, especially on public transport, similar to smoke-free areas for nonsmokers.*
- *Promote the development of communication technologies and electricity use that is more compatible with health. Prefer wired solutions for home use and public facilities. Expand fiberoptic networks as the foundation of a modern, sustainable, and performance-based technology that meets the ever-increasing demand for higher data transmission rates.*
- *Provide government funding for industry-independent research and education that do not dismiss strong scientific and medical findings of potential risks, but rather work to clarify those risks.*

We also call on you as an individual: Prefer wired communication technologies. Inform yourself and pass this information on to your family, neighbors, friends, and politicians. You can make a difference by sharing information and making precautionary choices so that the protection of human health and the environment is not left to and limited by commercial interests.”

American Academy of Pediatrics (July 2012)

The American Academy of Pediatrics (AAP), a non-profit professional organization of 60,000 primary care pediatricians, pediatric medical subspecialists, and pediatric surgical specialists in the

United States dedicated to the health, safety and well-being of infants, children, adolescents, and young adults strongly supports the proposal for a formal inquiry into radiation standards for cell phones and other wireless products. The Academy encourages the Federal Communications Commission (FCC) to vote to move forward with it's proposed inquiry into the adequacy of the existing FCC public safety limits (25 and Appendix D).

> *"The FCC has not assessed the standard for cell phone radiation since 1996. According to industry groups, approximately 44 million people had mobile phones when the standard was set; today, there are more than 300 million mobile phones in use in the United States. While the prevalence of wireless phones and other devices has sky-rocketed, the behaviors around cell phone uses have changed as well. The number of mobile phone calls per day, the length of each cell phone call, and the amount of time people use mobile phones has increased, while cell phone and wireless technology has undergone substantial changes. Many more people, especially adolescents and young adults, now use cell phones as their only phone line and they begin using wireless phones at much younger ages."*
>
> *"The AAP believes the inquiry to reassess the radiation standard presents an opportunity to review its impacts on children's health and well-being. In the past, such standards have generally been based on the impact of exposure on an adult male. Children, however, are not little adults and are disproportionately impacted by all environmental exposures, including cell phone radiation. In fact, according to IARC, when used by children, the average RF energy deposition is two times higher in the brain and 10 times higher in the bone marrow of the skull, compared with mobile phone use by adults. While the Academy appreciates that the FCC is considering investigating whether the emission standards should be different for devices primarily used by children, it is essential that any new standard for cell phones or other wireless devices be based on protecting the youngest and most vulnerable populations to ensure they are safeguarded throughout their lifetimes."*
>
> *"Finally, in reviewing the SAR standard, the FCC has the opportunity to highlight the importance of limiting media use among children. The Academy has found potentially negative effects and no known positive effects of media use by children under the age of two, including television, computers, cell phones, and other handheld wireless devices. In addition, studies consistently show that older children and adolescents utilize media at incredibly high rates, which potentially contributes to obesity and other health and developmental risks. In reviewing the SAR limit, the FCC has the opportunity to improve the health of our nation by highlighting the importance of limiting screen time and media use for children and adolescents."*

IV. LOCAL AND NATIONAL COUNTRY ACTIONS (2007 – 2012)

City of Brussels

The order of 1 March 2007 on the protection of the environment against the potentially harmful effects and nuisances caused by non-ionizing radiation, established a new regional framework legislation. Installations emitting electromagnetic radiation in the Brussels-Capital Region need environmental permits to be issued by Brussels Environment (26). The ordinance defines a standard of 3 V/m (also ~ 24 $\mathrm{mW/m^2}$ ~ 2.4 $\mu\mathrm{W/cm^2}$) is not exceeded by the transmitting mobile phone antennas. Compliance with this standard is applied since 14 March 2009.

“Environmental permit for antennas: The steps of the procedure (27)

1. *Introduction of the permit application*
   The application of the *environment permit is introduced by the operator of the antenna to Brussels Environment includes a technical dossier containing plans from a simulation of the electromagnetic field in a radius of influence of 200 meters from the transmitting antenna.*
   This simulation takes into account the technical characteristics of the antenna and the surrounding environment (presence of buildings ...). It aims to ensure that 25% of the 3 V/m standard (also ~ 24 mW/m^2 ~ 2.4 µW/cm^2) [given as power density = 1.5 V ~ 6 mW/m^2 ~ 0.6 µW/cm^2/m] *is not exceeded in any place accessible to the public.*
2. *Site visit and review of the record*
   A Brussels Environment agent reviews the application and conducts a site visit to see if the simulation is correct and if the environmental situation close to the antenna described in the application file corresponds reality given. If this is the case, the file is submitted to public inquiry.
3. *Public Inquiry*
   The application is submitted to a 15 days public inquiry to notify you and allow you to give your opinion. Public inquiry was announced by red posters usual affixed near the place of the antenna location. Any citizen can go to municipal services concerned to take note of the case.
4. *Decision*
   The environmental permit is granted or refused by Brussels Environment. This license ensures that all measures for safety and protection of the environment and residents are provided.”

Principality of Liechtenstein

In 2008 in the Principality of Liechtenstein a new environmental law came into effect including regulations and legal limits for cellular transmitters (28). The complete text for article 31 and 34 is given below. Article 31 defines locations with sensitive use where site specific limits have to be applied. However article 34, paragraph 4 (0.6 V/m limit) had been repealed in 2009 after business associations had initiated a national referendum (29).

Article 31 - Places of Sensitive Use

Regarded as places of sensitive use:

a) *rooms in buildings where people stay regularly over a long period;*
b) *playgrounds and rest places of schools, kindergartens and nursery schools operated by the public;*
c) *fixed outdoor workplaces where work-related to the same person is shown during more than 800 hours a year. Including, in particular fixed sales stands and Jobs at permanently installed equipment, but not outside areas of restaurants and construction sites;*
d) *those areas of undeveloped land in construction zones on which uses are permitted by letters a and b.*

Article 34 transmitters for cellular and wireless local loops

Site specific limits

1) *For transmitters of mobile cellular networks and transmitters for wireless local loops with a total effective radiated power of at least 6 watts, the site specific limits under paragraph 2 and 4 apply. They do not apply for radio relay systems, the wireless network security "Polycom" and other radio networks of security and rescue organizations.*
2) *The site specific limit for the effective value (rms) of the electric field strength is:*
   a) for installations transmitting exclusively in the frequency range of 900 MHz: 4.0 V/m (also =

42 mW/m² = 4.2 µW/cm²);
b) for facilities that broadcast exclusively in the frequency range of 1800 MHz or in a higher frequency range: 6.0 V/m (also ~ 100 mW/m² = 10 µW/cm²).;
c) for facilities that broadcast in both frequency ranges specified in letters a and b: 5.0 V/m (also = 66 mW/m² = 6.6 µW/cm²).

3) *Whereas the operative mode and the maximum call and data traffic is at maximum transmission power.*
4) *Holder of a broadcast system are required to reduce the actual electric filed strength to the lowest technically feasible value, using appropriate measures and to accomplish by the end of 2012 an actual electric field strength of 0.6 V/m (also ~ 1 mW/m² ~ 0,1 µW/cm²) on average.*
5) *The Government shall provide further details by ordinance.*

Italy – Autonomous Province of Bolzano - South Tyrol (2009)

In a Decree dated April 29, 2009, the governor of the Autonomous Province Bolzano issued Regulation No. 24 concerning telecommunications infrastructure. In the autonomous province of Bolzano radio- and cellular transmitter sites have to be operated that take health aspects into account (30, 31). In practice e.g. radio transmitters had been aggregated on tall mast sites preferably outside residential areas on mountains. The population exposure from cellular antenna sites is calculated with help of predictive software and the best possible sites are evaluated. Each site has to be approved by a communications commission. The national limit for the sum of all RF sources in Italy is 6 V/m (also ~ 100 mW/m² ~ 10 µW/cm²). In the autonomous province of Bolzano the competent authority - State Agency for Environment - negotiates each cellular site with the relevant operator(s) in order to achieve a site specific exposure of 3 V/m (also ~ 24 mW/m² ~ 2.4 µW/cm²) and lower (32).

Austria – Ministry of Health 2010

In December 2010 the document "Aspects of the current health assessment of mobile communications - Recommendation of the Supreme Health Council" was published (33). Some of the recommendations are listed below.

> *"... Radio equipment, which leads to a prolonged exposure of people should be set up using a precautionary target value, since long-term effects can not be excluded with sufficient certainty. This target value should be set for high-frequency effects at least a factor of 100 below the limit for the power density of the ÖNORM E 8850 (note by the author: similar to ICNIRP 1998). In addition, legal measures should be taken, that*
> *a) in case that various electromagnetic fields acting simultaneously, all relevant frequencies of different emitters are not to exceed the limits and*
> *b) operators are encouraged to minimize exposure from electromagnetic fields well below the limit values during planning and operation."*
>
> *„ ... In view of the many pending issues, the rational use of mobile phones should be taken generally, which seeks to have meaningful use and avoid unnecessary exposure. This is especially true for children and adolescents, since they will be predictable more exposed over their lifetime and*

> *the organ-specific exposure through anatomical and developmental differences in certain tissues may be higher than in adults."*

Nine specific recommendations were given by the Austrian Supreme Health Council:

1. *"If possible, do not call, when the reception is poor.*
2. *Keep calls short.*
3. *In situations where you can choose between mobile and fixed-line, use the landline.*
4. *Make calls in the car as little as possible.*
5. *With GSM (2 G) phones, wait a little time while connecting, before you run the phone to your head. Exposure by UMTS (3 G) mobile phones is usually much lower. Make sure to set the connection in multi-band-mobiles preferably via UMTS (3 G)*
6. *Use headsets or speakerphones.*
7. *When buying a cell phone mind low SAR values.*
8. *Wear the mobile not directly on the body.*
9. *Send an SMS instead of calling."*

France (2010)

In 2010 in France the Environmental Law some regulations concerning EMF issues had been supplemented (34, 35). Some excerpts are given below:

Article 183

- *Wireless terminals that are intended to be connected with a public telephone network may not be placed on the market without additional equipment, which allows to limit the exposure of the head during communication.*
- *The Higher Audiovisual Council shall ensure that the development of the sector of audiovisual communication goes along with an increased level of protection of the environment and the health of the population.*
- *Any advertising, about what aid whatsoever, with the direct aim to promote the sale, the provision or the use of a mobile phone by children under 14 is prohibited.*
- *The payment or free circulation of goods which contain a radio equipment and their use is specifically designed for children under six may be banned by decree of the Minister of Health, in order to avoid excessive exposure of children.*
- *Individuals who are responsible for the transport of electrical energy have to carry out a regular control of the electromagnetic fields, which are induced by power lines. The result of these measurements is to report annually to the French Agency for Sanitary Safety of environment and labor, which will publish them.*
- *In kindergarten (pre-), in the primary schools and in secondary schools (secondary) the use of a mobile phone is prohibited by a student during the entire lesson and at the designated places given in the house rules.*

Article 184

For any mobile telephone that is offered for sale [in France], the specific absorption rate is legible and in French. It must also provide a recommendation for the use of additional equipment, by means

> *of which the radio exposure of the head can be limited during the communication, as in the fifth Paragraph of point I of Article 183 of this law provided.*

Austria – Austrian Medical Association (2012)

In 2012 the Austrian Medical Association published the "Guideline of the Austrian Medical Association for the diagnosis and treatment of EMF-related health problems and illnesses (EMF syndrome)"(36). The guideline is recommended to doctors of all disciplines in Austria. The guideline says in part:

> *"There has been a sharp rise in unspecific, often stress-associated health problems that increasingly present physicians with the challenge of complex differential diagnosis. A cause that has been accorded little attention so far is increasing electrosmog exposure at home, at work and during leisure activities, occurring in addition to chronic stress in personal and working life. It correlates with an overall situation of chronic stress that can lead to burnout.*
>
> *How can physicians respond to this development?*
>
> *The Austrian Medical Association has developed a guideline for differential diagnosis and potential treatment of unspecific stress-related health problems associated with electrosmog. Its core element is a patient questionnaire consisting of a general assessment of stress symptoms and a specific assessment of electrosmog exposure. The guideline is intended as an aid in diagnosing and treating EMF-related health problems."*
>
> *Key elements of the guideline are:*
>
> 1. *History of health problems and EMF exposure*
> 2. *Examination and findings*
> 3. *Measurement of EMF exposure*
> 4. *Prevention or reduction of EMF exposure*
> 5. *Diagnosis*
> 6. *Treatment*

Russian National Committee on Non-Ionizing Radiation (2011 and 2012)

On March 3, 2011 the Russian National Commitee on Non-Ionizing Radiation Protection approved the "Resolution: Electromagnetic Fields from Mobile Phones: Health Effects on Children and Teenagers" (37 and Appendix E). Parts of the resolution are given below.

> *"The Resolution evolved from scientific statements adopted by RNCNIRP in 2001, 2004, 2007, 2008 and 2009, taking into account contemporary views and actual scientific data. The Resolution represents a viewpoint of the professional scientific community and is meant for public dissemination, for the consumers of the mobile telecommunications services, as well as for the legislative and executive authorities who develop and implement health protection, environmental, communication, scientific and safety policies."*

In 2012, the RCNIRP issued an update to this Resolution, calling on all countries to halt the use of wireless technologies in the school classrooms, and to move quickly to replace wireless with wired internet and teaching technologies (38 and Appendix F).

V. INTERNATIONAL HEALTH AGENCY ACTION

WHO International Agency for Research On Cancer – Formal Classification (2011)

On May 31, 2011 the WHO/International Agency for Research on Cancer (IARC) classified radiofrequency electromagnetic fields as possibly carcinogenic to humans (Group 2B), based on an increased risk for glioma, a malignant type of brain cancer, associated with wireless phone use (39, 40).

A group of 30 researchers, scientists and medical doctors were invited to participate in an assessment of the scientific literature on radiofrequency radiation carcinogenicity in Lyon, France. Under the auspices of IARC, this IARC Monograph Working Group on RFR conducted a comprehensive scientific assessment of RF studies and determined:

> *"In view of the limited evidence in humans and in experimental animals, the Working Group classified RF- EMF as "possibly carcinogenic to humans" (Group 2B). This evaluation was supported by a large majority of Working Group members."*
>
> *"The Working Group concluded that the (Interphone Final Report) findings could not be dismissed as reflecting bias alone, and that a causal interpretation between mobile phone RF-EMF exposure and glioma is possible. A similar conclusion was drawn from these two studies for acoustic neuroma, although the case numbers were substantially smaller than for glioma."*

It is important to recognize that the IARC RF Working Group did not find the evidence insufficient to classify (Group 3) or not a carcinogen (Group 4). Both of these possible outcomes to the scientific assessment could have rendered a substantially weaker conclusion. Where there has been the necessity of a virtual scientific paradigm shift to accommodate ANY consideration of both ELF-EMF and RFR to the status where legitimate scientific attention is achieved is a notable achievement. There is a very high bar set to show that non-chemical carcinogens warrant IARC carcinogenicity evaluation - it greatly exceeds that necessary for chemicals and other toxins.

The WHO press release No° 208 states

> *"The IARC Monograph Working Group discussed the possibility that these exposures might induce long-term health effects, in particular an increased risk for cancer. This has relevance for public health, particularly for users of mobile phones, as the number of users is large and growing,*

> *particularly among young adults and children."*

The corresponding monograph has not been published as of October 2012. On request, IARC clarified the frequency range covered by the monograph (41).

> *"The IARC Monographs classification of Radiofrequency Electromagnetic Fields (RF-EMF) covers the entire radiofrequency segment of the electromagnetic spectrum (30 kHz-300 GHz). Within this spectrum, the electromagnetic fields around (or the radiation emitted by) mobile telephones represent the most intense and most wide-spread exposure situation, for which a small increase in risk for glioma and acoustic neuroma has been found in the group of 'heavy users'. Other devices that emit the same type of RF radiation - base-station antennas, radio/tv antennas, WiFi stations, smart meters - fall under the same evaluation. However, because the exposure levels for many of these other devices and exposure situations are so much lower than the exposure to someone who has a functioning cell phone against her/his ear, the risk will be considerably less (although the hazard still exists)."*

VI. CONCLUSIONS

1) The European Environmental Agency (2007) concludes that: "*(T)here are many examples of the failure to use the precautionary principle in the past, which have resulted in serious and often irreversible damage to health and environments. Appropriate, precautionary and proportionate actions taken now to avoid plausible and potentially serious threats to health from EMF are likely to be seen as prudent and wise from future perspectives. We must remember that precaution is one of the principles of EU environmental policy*."

2) The European Parliament, the Council of Europe and various governmental agencies in Europe, Scandinavia, Israel, North America, India and Asia have called for better warnings, to reduce or eliminate exposures from wireless devices, to label devices with health warnings, to develop new, lower public safety standards, to protect sensitive subgroups (children, people who are sensitized to EMF and wireless radiation already (electrosensitivity), and to inform and protect pregnant women and their young from unnecessary exposures. The countries of France, Italy, Belgium, the Principality of Liechtenstein, Switzerland, Austria, the United Kingdom, and others have led in proposing new restrictions on wireless exposures, based on scientific and public health reviews of the evidence. The US Government Accountability Office has called for review of American (FCC) safety limits for wireless devices.

3) Physicians and health advisory groups around the world have called for prudent public health actions that include reducing or eliminating ELF and RFR exposures, especially for pregnant women and for the developing fetus, and children, and particularly where other options are available (in the case of wireless exposures in particular). Some of these groups include the Austrian Ministry of Health, the Russian National Committee on Non-Ionizing Radiation, the American Academy of Environmental Medicine, the American Academy of Pediatrics, the British Chief Medical Officer, and many more governmental agencies across Europe, Scandinavia, North America, India and Asia.

4) Physicians and researchers who have published in-depth reviews on the science and public health policy implications of ELF and RFR risks to health include Pathophysiology, Vol 16 (2,3); 2009; the two-volume *Non Thermal effects and Mechanisms of interaction between Electromagnetic Fields and Living Matter*. eds Giuliani L and Soffritti, M, ICEMS, Ramazzini Institute, Bologna, Italy., 2010; the World Health Organization INTERPHONE Final Report, 2010; and the WHO International Agency for Research on Cancer RFR Monograph (Baan et al, 2011) designating RFR

as a Group 2B Possible Human Carcinogen.

5) Overall, these provide support for warnings and advice to consumers and the public that the body of evidence for bioeffects from daily exposure levels of ELF and RFR can reasonably be presumed to result in adverse health impacts with chronic exposure. The studies on which these warnings rely establish that bioeffects from exposure to ELF and RFR are established, not speculative or weak. Further, they establish that existing ICNIRP and FCC public safety limits are inadequate to protect public health; and underscore the need for new, biologically-based public exposure standards.

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VIII. APPENDICES

APPENDIX A Full Text of European Parliament Statement - 2008

"The European Parliament ,

– having regard to the Communication from the Commission to the Council, the European Parliament and the European Economic and Social Committee on the mid-term review of the European Environment and Health Action Plan 2004-2010 (COM(2007)0314),

– having regard to its resolution of 23 February 2005 on the European Environment and Health Action Plan 2004-2010(1) ,

– having regard to the World Health Organisation (WHO) report of 27 July 2007 entitled 'Principles for evaluating health risks in children associated with exposure to chemicals',

– having regard to Articles 152 and 174 of the EC Treaty targeting a high level of protection for human health and the environment,

– having regard to Decision No 1350/2007/EC of the European Parliament and of the Council of 23 October 2007 establishing a second programme of Community action in the field of health (2008-13)(2) ,

– having regard to Rule 45 of its Rules of Procedure,

– having regard to the report of the Committee on the Environment, Public Health and Food Safety (A6-0260/2008),

A. noting with interest the fact that, since 2003, the EU has based its health-protection policy on closer cooperation between the health, environment and research sectors, so that it may be hoped that a coherent and integrated European environmental health strategy will eventually be introduced,

B. whereas the courses of action currently being followed by the EU as part of its first environment and health action plan (2004-2010) (COM(2004)0416) - namely, the preparation of indicators, the development of integrated monitoring, the collection and evaluation of relevant data as well as an increase in the volume of research - will allow greater insight into the interactions between sources of pollution and health effects but are known to be inadequate as a means of reducing the growing number of diseases related to environmental factors,

C. whereas it is virtually impossible to establish a mid-term assessment of the aforementioned action plan, since the latter pursues no clear, quantified objective and the total budget allocated to it is difficult to determine and definitely insufficient for its efficient promotion,

D. whereas the main objective of the 2008-2013 health programme is to act upon the factors which traditionally determine health (diet, smoking, alcohol consumption and the use of drugs); whereas this 2004-2010 action plan should focus on certain new health challenges and in addition address the determining environmental factors which affect human health, such as indoor and outdoor air quality, electromagnetic waves, nanoparticles and chemicals which are a cause for serious concern (substances classed as carcinogenic, mutagenic or toxic to reproduction [CMR], endocrine disruptors), as well as risks to health arising from climate change,

E. whereas respiratory illnesses rank second as a cause of death and in terms of incidence, prevalence and cost within the EU, whereas they constitute the main cause of death amongst children under the age of five *and whereas such diseases are continuing to progress on account of - in particular - indoor and outdoor air pollution,*

F. whereas atmospheric pollution caused, in particular, by fine particles and ground-level ozone, is a significant threat to human health which is affecting the proper development of children and reducing life expectancy in the EU(3) ,

G. whereas, with reference to the issue of urban environmental health, particularly the quality of indoor air, the Community - in accordance with the subsidiarity and proportionality principles - should do more to combat domestic pollution, since Europeans spend on average 90% of their time inside buildings,

H. whereas at the 2004 and 2007 WHO ministerial conferences on health and the environment, attention was drawn to the links between the complex combined influence of chemical pollutants and a number of chronic illnesses and disorders (affecting children in particular); whereas those concerns are also expressed in official documents issued in connection with the United Nations Environment Programme (UNEP) and by the Intergovernmental Forum on Chemical Safety (IFCS),

I. whereas there is increasing scientific evidence that certain cancers, such as cancer of the bladder, bone cancer, lung cancer, skin cancer, breast cancer and others are caused not only by the effects of chemical substances, radiation and airborne particles but also by other environmental factors,

J. whereas these problematic developments in environmental health have been accompanied in recent years by the emergence of new diseases or syndromes, such as multiple chemical hypersensibility, dental-amalgam syndrome, hypersensitivity to electromagnetic radiation, sick-building syndrome and attention-deficit and hyperactivity syndrome in children,

K. whereas the precautionary principle has been enshrined in the Treaty since 1992 and whereas the European Court of Justice has repeatedly specified the substance and the scope of that principle in Community law, which constitutes one of the cornerstones of the protection policy pursued by the Community in the field of health and the environment(4) ,

L. having regard to the highly restrictive - if not to say impracticable - nature of the criteria adopted by the Commission in its 2 February 2000 Communication on the precautionary principle (COM(2000)0001),

M. having regard to the importance of human biological monitoring as a tool for assessing the European population's degree of exposure to the effects of pollution and the determination (repeatedly expressed by Parliament in Paragraph 3 of its aforementioned resolution of 23 February 2005 and in the conclusions issued at the end of the 20 December 2007 Council meeting of Environment Ministers) to expedite the introduction of a biological-monitoring programme at EU level,

N. whereas it is readily acknowledged that climate change can play an important role in increasing the severity and incidence of certain diseases and in particular that heat-wave frequency, flooding and wildfires as the most frequent natural disasters in the EU can lead to additional diseases, poor sanitation and deaths, while at the same time recognising the beneficial effects on health of measures to alleviate climate change,

O. whereas climate change will have significant effects on human health, inter alia by encouraging the development of certain infectious and parasitic diseases mainly because of changes in temperature and humidity and their impact on ecosystems, animals, plants, insects, parasites, protozoa, microbes and viruses,

P. whereas Directive 2000/60/EC of the European Parliament and of the Council of 23 October 2000 establishing a framework for Community action in the field of water policy(5) *and its daughter directives contain clear provisions concerning the preservation and restoration of healthy waters,* *Q. whereas environmental medicine is a new medical discipline based on university teaching which is still too fragmentary and unevenly distributed amongst the Member States and which thus deserves to be supported and promoted within the EU,*

R. whereas the number of persons suffering as a result of environmental factors is increasing and epidemiologies should be developed in order to obtain a full picture of diseases which are caused wholly or in part by environmental factors,

1. Acknowledges the efforts made by the Commission since the action plan was launched in 2004, particularly in terms of improving the chain of information concerning health and the environment, integrating and expanding European research in this area and cooperating with specialist international organisations such as the WHO;

2. Considers, however, that such an action plan is bound to fail at least in part, since it is designed solely to accompany existing Community policies, it is not based upon a preventive policy intended to reduce illnesses linked to environmental factors, and it pursues no clear, quantified objective;

3. Draws the Commission's attention to the fact that a programme has already been carried out under the aegis of the WHO as part of which the WHO Member States established their own national and local environmental health action plans comprising specific objectives and implementation plans; recommends to the Commission therefore that it review this WHO programme as a possible model which could also serve as a useful example to the Union in the future;

4. Deeply regrets the fact that the Commission (and in particular its Research DG) has not provided sufficient funding for human biological monitoring in 2008 to enable it (as it had promised Parliament and the Member States) to introduce a consistent approach to biological monitoring within the EU;

5. Calls upon the Commission to respond by 2010 to two essential objectives which the Commission set itself in 2004 and to establish and carry out a practicable communication strategy for these objectives, namely to make members of the general public aware of environmental pollution and the impact thereof on their health, and to reconsider and adapt European risk-reduction policy;

6. Strongly recommends that the Commission and Member States meet their obligations as regards implementation of Community legislation;

7. Stresses that, when it comes to assessing the impact of environmental factors on health, consideration should be given first and foremost to vulnerable groups such as pregnant women, newborn babies, children and the elderly;

8. Calls for special attention to be given to vulnerable groups, who are the most susceptible to pollutants, by introducing measures to reduce exposure to indoor environmental contaminants in healthcare facilities and schools through the adoption of sound indoor air quality management practices;

9. Urges the Commission, when drafting proposals for the revision of existing laws, not to weaken those laws under pressure from lobbies or regional or international organisations;

10. Points that the EU needs to apply a continuous dynamic and flexible approach to the Action Plan; considers that it is therefore of paramount importance to acquire specific expertise on the subject of environmental health, to be based on transparency and on a multidisciplinary and adversarial approach which would thus enable the general public's distrust of official agencies and committees of experts to be countered; points to the importance of improving the training of health experts by means, in particular, of exchanges of best practice at Community level;

11. Points out that in recent years there have been genuine advances in environmental policy in the form of (for example) a reduction in air pollution, an improvement in water quality, the collection and recycling of waste, the monitoring of chemicals and a ban on leaded petrol, but notes at the same time that EU policy still lacks a comprehensive preventive strategy and fails to apply the precautionary principle;

12. Calls, therefore, on the Commission to revise the criteria laid down in its aforementioned Communication as regards recourse to the precautionary principle pursuant to European Court of Justice case-law, in order to ensure that an action and security principle based on the adoption of provisional and proportionate measures lies at the heart of Community health and environment policies;

13. Considers that shifting the burden of proof onto producers or importers and requiring them to demonstrate that a product is harmless would make it possible for a policy based on prevention to be promoted (as provided for in European Parliament and Council Regulation (EC) No 1907/2006 of 18 December 2006 concerning the registration, evaluation, authorisation and restriction of chemicals (REACH) and establishing a European Chemicals Agency(6)), and encourages the Commission to extend that obligation to Community legislation concerning all products; considers that any increase in animal testing under the Action Plan should be avoided and full regard should be paid to the development and use of alternative methods;

14. Calls once again upon the Commission to come forward as soon as possible with concrete measures on indoor air quality which would ensure a high level of protection of health and safety indoors to be established, in particular when revising Council Directive 89/106/EEC of 21 December 1988 on the approximation of laws, regulations and administrative provisions of the Member States relating to construction products(7) , and to propose measures to increase the energy efficiency of buildings and the safety and the harmlessness of chemical compounds used in equipment and furnishings;

15. Recommends that, in order to reduce damaging effects of the environment on health, the Commission should call upon Member States, by means of tax concessions and/or other economic incentives, to interest market operators in improving the quality of indoor air and reducing exposure to electromagnetic radiation in their buildings, branch establishments and offices;

16. Recommends that the Commission draft appropriate minimum requirements to guarantee the quality of indoor air in buildings to be newly built;

17. Recommends that, in awarding individual European Union support, the Commission bear in mind its impact on the quality of indoor air, exposure to electromagnetic radiation and the health of particularly endangered sections of the population in the projects concerned in a similar way to that in which attention is devoted to environmental protection criteria;

18. Calls for environmental quality standards for priority substances in water to be laid down in accordance with the latest scientific knowledge and regularly brought into line with current scientific thinking;

19. Points out that certain Member States have successfully introduced mobile analysis laboratories (or "green ambulances") to enable habitat pollution in public and private places to be diagnosed swiftly and reliably; considers that the Commission could promote such a practice within the Member States which have not yet acquired such a means of direct intervention at a polluted site;

20. Is concerned about the lack of specific legal provisions to ensure the safety of consumer products containing nanoparticles and the relaxed attitude of the Commission with regard to the need to review the regulatory framework for the use of nanoparticles in consumer products in light of the increasing number of consumer products containing nanoparticles being put on the market;

21. Is greatly concerned at the Bio-Initiative international report(8) concerning electromagnetic fields, which summarises over 1500 studies on that topic and which points in its conclusions to the health risks posed by emissions from mobile-telephony devices such as mobile telephones, UMTS, Wifi, Wimax and Bluetooth, and also DECT landline telephones;

22. Notes that the limits on exposure to electromagnetic fields which have been set for the general public are obsolete, since they have not been adjusted in the wake of Council Recommendation 1999/519/EC of 12 July 1999 on the limitation of exposure of the general public to electromagnetic fields (0Hz to 30 GHz)(9) , obviously take no account of developments in information and communication technologies, of the recommendations issued by the European Environment Agency or of the stricter emission standards adopted, for example, by Belgium, Italy and Austria, and do not address the issue of vulnerable groups, such as pregnant women, newborn babies and children;

23. Calls, consequently, upon the Council to amend its Recommendation 1999/519/EC in order to take into account the Member States' best practices and thus to set stricter exposure limits for all equipment which emits electromagnetic waves in the frequencies between 0.1 MHz and 300 GHz;

24. Takes a very serious view of the multiple health risks created by global warming on EU territory and calls for enhanced cooperation between the WHO, the Member States" monitoring authorities, the Commission and the European Centre for Disease Prevention and Control in order to bolster the early-warning system and thus to curb the harmful effects which climate change has on health;

25. Highlights that this Action Plan would benefit from being extended to cover negative impacts of climate change on human health by elaborating on effective adaptation measures necessary at Community level, such as:

- *systematic public education programmes and awareness-raising;*
- *integration of climate change adaptation measures into public health strategies and programmes, such as communicable and non-communicable diseases, workers' health and animal diseases hazardous to health;*
- *proper surveillance aiming at the early detection of disease outbreaks;*
- *health-related early warning systems and response;*
- *coordination of existing environmental data monitoring networks with disease outbreak networks;*

26. Calls on Member States and the Commission to respond adequately to the new threats posed by climate change such as the increased presence of emerging viruses and undetected pathogens and therefore implement new existing pathogen reduction technologies that reduce known and undetected viruses and other pathogens transmitted by blood;

27. Regrets that the current cost benefit impact assessment of the '20 20 by 2020 Europe's Climate Change Opportunity' (COM(2008)0030) only considers the health benefits of reduced air pollution at a 20% reduction of greenhouse gas emissions by 2020; calls on the Commission to ensure that the (ancillary) co-benefits to health of various levels of ambition, in line with the International Panel on Climate Change recommendations of domestic 25% to 40% as well as possibly 50% or more of greenhouse gas emission reduction by 2020, are urgently investigated and modelled into an impact assessment by the Commission;

28. Calls on the Commission to pay attention to the serious problem of mental health, considering the number of suicides in the EU, and to devote more resources to the development of adequate prevention strategies and therapies;

29. Reiterates that the Commission and the Member States should support the WHO Children's Environment and Health Action Plan in Europe, to encourage it both through EU and bilateral development policy, and to encourage similar processes outside the WHO Europe Region;

30. Calls on the Commission to reincorporate into its second action plan the initiative SCALE (Science, Children, Awareness, Legal instruments, Evaluation) relating to the reduction of exposure to pollution, as set out in the European Environment and Health Strategy (COM(2003)0338);

31. Urges the Commission to work on and provide instruments that would foster the development and promotion of innovative solutions, as stressed within the Lisbon agenda framework, in order to minimise major health risks from environmental stressors;

32. Urges the Council to take a decision without delay on the proposal for a regulation establishing the Union Solidarity Fund, as Parliament adopted its position as long ago as 18 May 2006(10) ; considers that the new regulation, which, together with other measures, will lower thresholds for the entry into force of the Union Solidarity Fund, will make it possible to alleviate more effectively, flexibly and quickly damage caused by natural or man-made disasters; stresses that such a financial instrument is very important, particularly because it is assumed that natural disasters will occur more frequently in future, partly on account of climate change;

33. Recommends, as SMEs are of decisive economic importance in Europe, that the Commission should provide technical support to SMEs to make it possible, and help them, to comply with binding environmental health regulations and encourage them to make other changes which are positive from the point of view of environmental health and affect the operation of enterprises;

34. Advises the Commission to envisage (by 2010 and under the "second cycle" of the health and environment action plan) refocusing its initiatives on vulnerable populations and to devise new methods of risk assessment, taking into account the fundamental fact that children, pregnant women and older people are particularly vulnerable;

35. Urges the Commission and Member States therefore to acknowledge the advantages of the prevention and precautionary principles and to develop and implement tools enabling potential environmental and health threats to be anticipated and countered; recommends that the Commission cost the 'second cycle' of this action plan and make provision for appropriate funding covering a larger number of practical measures to reduce environmental impact on health and to implement prevention and precautionary measures;

36. Instructs its President to forward this resolution to the Council, the Commission, the governments and parliaments of the Member States and the WHO.

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(1) *OJ C 304 E, 1.12.2005, p. 264.*

(2) *OJ L 301, 20.11.2007, p. 3.*

(3) *Europe's environment, the fourth assessment, summary, European Environment Agency (10.10.2007).*

(4) *Judgment of 23 September 2003 in Case C-192/01, Commission/Denmark, ECR 2003, p. I-9693; judgment of 7 September 2004 in Case C-127/02, Landelijke Vereniging tot Behoud van de Waddenzee and Nederlandse Vereniging tot Bescherming van Vogels, ECR 2004, p. I-7405.*

(5) *OJ L 327, 22.12.2000, p. 1.*

(6) *OJ L 396, 30.12.2006, p. 1; corrected version in OJ L 136, 29.5.2007, p. 3.*

(7) *OJ L 40, 11.2.1989, p. 12.*

(8) *Published by a group of independent scientists on 31 August 2007. For details, see: www.bioinitiative.org.*

(9) *OJ L 199, 30.7.1999, p. 59.*

(10) *OJ C 297 E, 7.12.2006, p. 331.*

APPENDIX B Full Text of European Parliament Resolution – 2009

European Parliament 2009

On April 2, 2009, the European Parliament adopted the "European Parliament resolution of 2 April 2009 on health concerns associated with electromagnetic fields (2008/2211(INI))" (10). The Document was based on the "Report on health concerns associated with electromagnetic fields", Rapporteur: Frederique Ries (11) Committee on the Environment, Public Health and Food Safety.

A. whereas electromagnetic fields (EMFs) exist in nature and have consequently always been present on earth; whereas, however, in recent decades, environmental exposure to man-made sources of EMFs has risen constantly, driven by demand for electricity, increasingly more specialised wireless technologies, and changes in the organisation of society; whereas the end effect is that every individual is now being exposed to a complex mixture of electric and magnetic fields of different frequencies, both at home and at work,

B. whereas wireless technology (mobile phones, Wi-Fi/WiMAX, Bluetooth, DECT landline telephones) emits EMFs that may have adverse effects on human health,

C. whereas most European citizens, especially young people aged from 10 to 20, use a mobile phone, an object serving a practical purpose and as a fashion accessory, and whereas there are continuing uncertainties about the possible health risks, particularly to young people whose brains are still developing,

D. whereas the dispute within the scientific community regarding the potential health risks arising from EMFs has intensified since 12 July 1999, when exposure limits for fields in the 0 Hz to 300 GHz range were laid down in Recommendation 1999/519/EC,

E. whereas the fact that the scientific community has reached no definite conclusions has not prevented some national or regional governments, in China, Switzerland, and Russia, as well as in at least nine EU Member States, from setting what are termed "preventive" exposure limits, that is to say, lower than those advocated by the Commission and its independent scientific committee, the Scientific Committee on Emerging and Newly Identified Health Risks(7) ,

F. whereas actions to limit the exposure of the general public to EMFs should be balanced against improvements to quality of life, in terms of safety and security, brought about by devices transmitting EMFs,

G. whereas among the scientific projects arousing both interest and controversy is the Interphone epidemiological study, financed by an EU contribution of EUR 3 800 000, primarily under the Fifth RTD Framework Programme(8) , the findings of which have been awaited since 2006,

H. whereas, however, there are some points that appear to be the subject of general agreement, in particular the idea that reactions to microwave exposure vary from one person to another, the need, as a matter of priority, to conduct exposure tests under actual conditions in order to assess the non-thermal effects associated with radio-frequency (RF) fields, and the fact that children exposed to EMFs are especially vulnerable(9) ,

I. whereas the EU has laid down exposure limits to protect workers from the effects of EMFs; whereas on the basis of the precautionary principle such measures should also be taken for the sections of population concerned, such as residents and consumers, *J. whereas the Special Eurobarometer report on Electromagnetic Fields (No 272a of June 2007) indicates that the majority of citizens do not feel that the public authorities inform them adequately on measures to protect them from EMFs,*

K. whereas it is necessary to continue investigations into intermediate and very low frequencies so that conclusions can be drawn as to their effects on health,

L. whereas the use of Magnetic Resonance Imaging (MRI) must not be threatened by Directive 2004/40/EC as MRI technology is at the cutting edge of research, diagnosis and treatment of life-threatening diseases for patients in Europe,

M. whereas the MRI safety standard IEC/EN 60601-2-33 establishes limit values for EMFs which have been set so that any danger to patients and workers is excluded.

1. Urges the Commission to review the scientific basis and adequacy of the EMF limits as laid down in Recommendation 1999/519/EC and report to the Parliament; calls for the review to be undertaken by the Scientific Committee on Emerging and Newly Identified Health Risks;

2. Calls for particular consideration of biological effects when assessing the potential health impact of electromagnetic radiation, especially given that some studies have found the most harmful effects at lowest levels; calls for active research to address potential health problems by developing solutions that negate or reduce the pulsating and amplitude modulation of the frequencies used for transmission;

3. Maintains that as well as, or as an alternative to, amending European EMFs limits, the Commission, working in coordination with experts from Member States and the industries concerned (electricity companies, telephone operators and manufacturers of electrical appliances including mobile phones), should draw up a guide to available technology options serving to reduce exposure to EMFs;

4. Notes that industry stakeholders as well as relevant infrastructure managers and competent authorities can already influence certain factors, for example setting provisions with regards to the distance between a given site and the transmitters, the height of the site in relation to the height of the base station, or the direction of a transmitting antenna in relation to living environments, and, indeed, should obviously do so in order to reassure, and afford better protection to, the people living close to such facilities; calls for optimal placement of masts and transmitters and further calls for the sharing of masts and transmitters placed in this way by providers so as to limit the proliferation of poorly positioned masts and transmitters; calls on the Commission and Member States to draw up appropriate guidance;

5. Invites the Member States and local and regional authorities to create a one-stop shop for authorisation to install antennas and repeaters, and to include among their urban development plans a regional antenna plan

6. Urges the authorities responsible for authorising the siting of mobile telephony antennas to reach agreement, jointly with the operators in that sector, on the sharing of infrastructure, in order to reduce the volume thereof and the exposure of the public to EMFs;

7. Acknowledges the efforts of mobile communications and other EMF-transmitting wireless technologies to avoid damaging the environment, and in particular to address climate change;

8. Considers that, given the increasing numbers of legal actions and measures by public authorities having the effect of a moratorium on the installation of new EMF-transmitting equipment, it is in the general interest to encourage solutions based on negotiations involving industry stakeholders, public *authorities, military authorities and residents" associations to determine the criteria for setting up new GSM antennas or high-voltage power lines, and to ensure at least that schools, crèches, retirement homes, and health care institutions are kept clear, within a specific distance determined by scientific criteria, of facilities of this type;*

9. Calls on the Member States to make available to the public, jointly with the operators in the sector, maps showing exposure to high-voltage power lines, radio frequencies and microwaves, and especially those generated by telecommunications masts, radio repeaters and telephone antennas. Calls for that information to be displayed on an internet page so that it can easily be consulted by the public, and for it to be disseminated in the media;

10. Proposes that the Commission consider the possibility of using funding from the Trans-European Energy Networks to investigate the effects of EMFs at very low frequencies, and particularly in electrical power lines,

11. Calls on the Commission, during the 2009-2014 parliamentary term, to launch an ambitious programme to gauge the electromagnetic compatibility between waves created artificially and those emitted naturally by the human body with a view to determining whether microwaves might ultimately have undesirable consequences for human health;

12. Calls on the Commission to present a yearly report on the level of electromagnetic radiation in the EU, its sources, and actions taken in the EU to better protect human health and the environment;

13. Calls on the Commission to find a solution enabling Directive 2004/40/EC to be implemented more rapidly and thus ensure that workers are properly protected against EMFs, just as they are already protected under two other Community acts against noise(10) and vibration(11) and to introduce a derogation for MRI under Article 1 of that Directive.

14. Deplores the fact that, as a result of repeated postponements since 2006, the findings of the Interphone study have yet to be published, the purpose of this international epidemiological study being to establish whether there is a link between use of mobile phones and certain types of cancer, including brain, auditory nerve, and parotid gland tumours;

15. Draws attention in this context to the appeal for caution from the coordinator of the Interphone study, Elisabeth Cardis, who, in the light of existing knowledge, recommends, as far as children are concerned, that mobile phones should not be used beyond reasonable limits and that landlines should be preferred;

16. Believes in any event that it is up to the Commission, which has an important contribution to the financing of this global study, to ask those in charge of the project why no definitive findings have been published and, should it receive an answer, to inform Parliament and the Member States without delay;

17. Also suggests to the Commission, to make for efficiency in policy and budget terms, that the Community funding earmarked for studies on EMFs be partly switched to finance a wide-ranging awareness campaign to familiarise young Europeans with good mobile phone techniques, such as the use of hands-free kits, keeping calls short, switching off phones when not in use (such as when in classes) and using phones in areas that have good reception;

18. Considers that such awareness-raising campaigns should also familiarise young Europeans with the health risks associated with household devices and the importance of switching off devices rather than leaving them on stand-by;

19. Calls on the Commission and Member States to increase research and development funding for the evaluation of potential long-term adverse effects of mobile telephony radio frequencies; calls also for an increase in public calls for proposals for investigation of the harmful effects of multiple exposure to different sources of EMFs, particularly where children are concerned;

20. Proposes that the European Group on Ethics in Science and New Technologies be given the additional task of assessing scientific integrity in order to help the Commission forestall possible cases of risk, conflict of interests, or even fraud that might arise now that competition for researchers has become keener;

21. Calls on the Commission, in recognition of the public concern in many Member States, to work with all relevant stakeholders, such as national experts, non-governmental organisations and industrial sectors, to improve the availability of, and access to, up-to-date information understandable to non-specialists on wireless technology and protection standards;

22. Calls on the International Commission on Non-Ionising Radiation Protection and the World Health Organisation (WHO) to be more transparent and open to dialogue with all stakeholders in standard setting;

23. Condemns certain particularly aggressive marketing campaigns by telephone operators in the run-up to Christmas and other special occasions, including for example the sale of mobile phones designed solely for children or free call time packages aimed at teenagers;

24. Proposes that the EU's indoor air quality policy should encompass the study of "wireless" domestic appliances, which, like Wi-Fi for Internet access and digital enhanced cordless telecommunications (DECT) telephones, have been widely adopted in recent years in public places and in the home, with the result that citizens are being continuously exposed to microwave emissions;

25. Calls, given its constant concern to improve consumer information, for the technical standards of the European Committee for Electrotechnical Standardisation to be amended with a view to imposing labelling requirements whereby the transmitting power would have to be specified and every wireless-operated device accompanied by an indication that it emitted microwaves;

26. Calls on the Council and Commission, in coordination with the Member States and the Committee of the Regions, to encourage the introduction of a single standard designed to ensure that local residents are subjected to as low a degree of exposure as possible when high-voltage grids are extended;

27. Is greatly concerned about the fact that insurance companies are tending to exclude coverage for the risks associated with EMFs from the scope of liability insurance policies, the implication clearly being that European insurers are already enforcing their version of the precautionary principle;

28. Calls on Member States to follow the example of Sweden and to recognise persons that suffer from electrohypersensitivity as being disabled so as to grant them adequate protection as well as equal opportunities;

29. Instructs its President to forward this resolution to the Council, the Commission, the governments and parliaments of the Member States, the Committee of the Regions, and the WHO.

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(1) OJ L 199, 30.7.1999, p. 59.

(2) OJ L 159, 30.4.2004, p. 1.

(3) OJ L 91, 7.4.1999, p. 10.

(4) OJ L 374, 27.12.2006, p. 10.

(5) Texts adopted, P6_TA(2008)0410.

(6) OJ C 175, 21.6.1999, p. 129.

(7) Opinion of 21 March 2007 adopted at the 16th plenary meeting of the Committee.

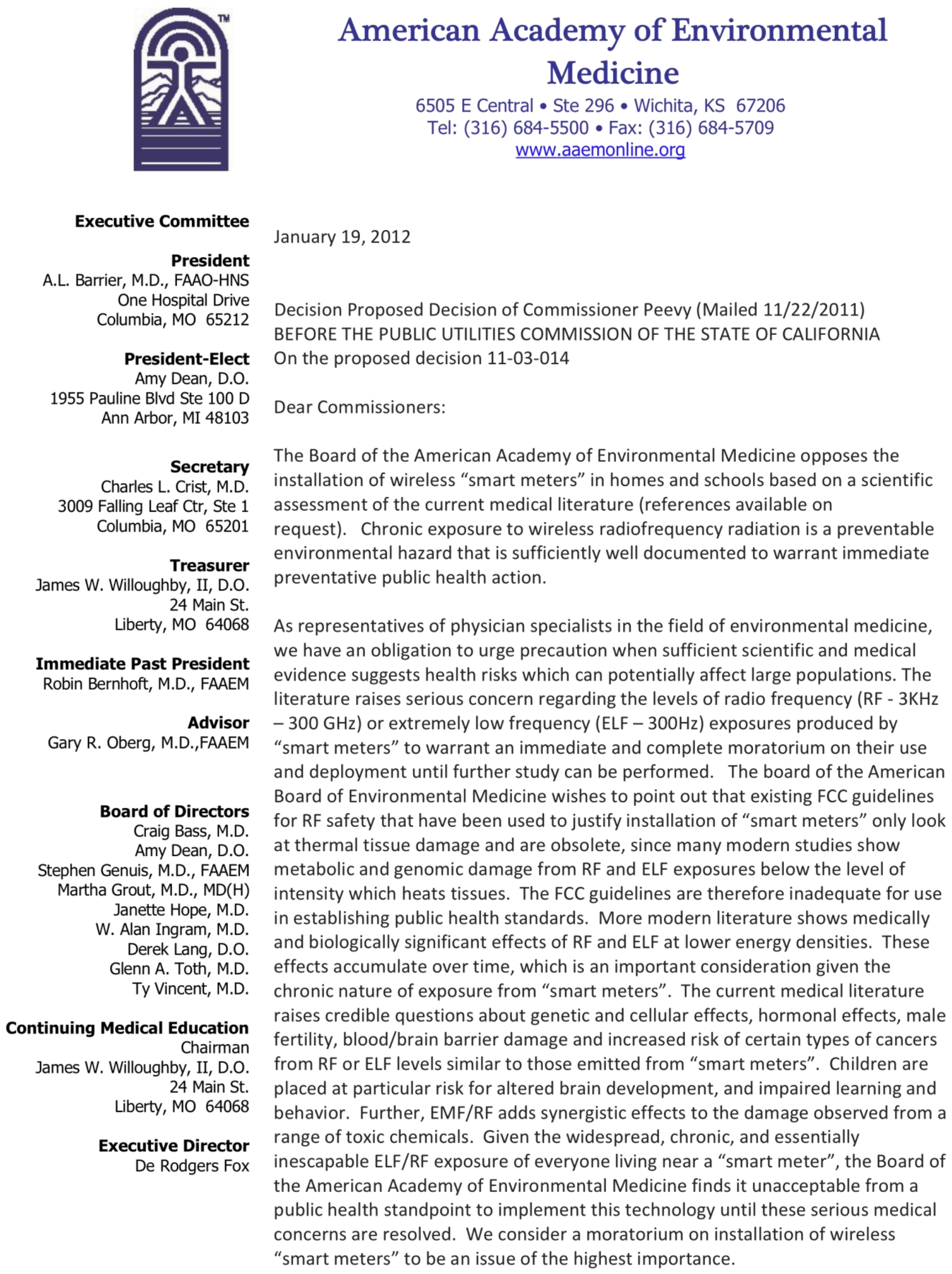
(8) Quality of life programme, contract No QLK4-1999-01563.

(9) March 2001 STOA study on "The physiological and environmental effects of non-ionising EMR", PE297.574.

(10) Directive 2003/10/EC of the European Parliament and of the Council of 6 February 2003 on the minimum health and safety requirements regarding the exposure of workers to the risks arising from physical agents (noise) (OJ L 42, 15.2.2003, p. 38).

(11) Directive 2002/44/EC of the European Parliament and of the Council of 25 June 2002 on the minimum health and safety requirements regarding the exposure of workers to the risks arising from physical agents (vibration) (OJ L 177, 6.7.2002, p. 13).

APPENDIX C American Academy of Environmental Medicine



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January 19, 2012

Decision Proposed Decision of Commissioner Peevy (Mailed 11/22/2011)
BEFORE THE PUBLIC UTILITIES COMMISSION OF THE STATE OF CALIFORNIA
On the proposed decision 11-03-014

Dear Commissioners:

The Board of the American Academy of Environmental Medicine opposes the installation of wireless “smart meters” in homes and schools based on a scientific assessment of the current medical literature (references available on request). Chronic exposure to wireless radiofrequency radiation is a preventable environmental hazard that is sufficiently well documented to warrant immediate preventative public health action.

As representatives of physician specialists in the field of environmental medicine, we have an obligation to urge precaution when sufficient scientific and medical evidence suggests health risks which can potentially affect large populations. The literature raises serious concern regarding the levels of radio frequency (RF - 3KHz – 300 GHz) or extremely low frequency (ELF – 300Hz) exposures produced by “smart meters” to warrant an immediate and complete moratorium on their use and deployment until further study can be performed. The board of the American Board of Environmental Medicine wishes to point out that existing FCC guidelines for RF safety that have been used to justify installation of “smart meters” only look at thermal tissue damage and are obsolete, since many modern studies show metabolic and genomic damage from RF and ELF exposures below the level of intensity which heats tissues. The FCC guidelines are therefore inadequate for use in establishing public health standards. More modern literature shows medically and biologically significant effects of RF and ELF at lower energy densities. These effects accumulate over time, which is an important consideration given the chronic nature of exposure from “smart meters”. The current medical literature raises credible questions about genetic and cellular effects, hormonal effects, male fertility, blood/brain barrier damage and increased risk of certain types of cancers from RF or ELF levels similar to those emitted from “smart meters”. Children are placed at particular risk for altered brain development, and impaired learning and behavior. Further, EMF/RF adds synergistic effects to the damage observed from a range of toxic chemicals. Given the widespread, chronic, and essentially inescapable ELF/RF exposure of everyone living near a “smart meter”, the Board of the American Academy of Environmental Medicine finds it unacceptable from a public health standpoint to implement this technology until these serious medical concerns are resolved. We consider a moratorium on installation of wireless “smart meters” to be an issue of the highest importance.

The Board of the American Academy of Environmental Medicine also wishes to note that the US NIEHS National Toxicology Program in 1999 cited radiofrequency radiation as a potential Carcinogen. Existing safety limits for pulsed RF were termed 'not protective of public health' by the Radiofrequency Interagency Working Gropu (a federal interagency working group includeing the FDA, FCC, OSHA, the EPA and others). Emissions given off by 'smart meters' have been classified by the World Health Organization International Agency for Research on Cancer (IARC) as a Possible Human Carciongen.

Hence, we call for:

- An immediate moratorium on "smart meter" installation until these serious public health issues are resolved. Continuing with their installation would be extremely irresponsible.
- Modify the revised proposed decision to include hearings on health impact in the second proceedings, along with cost evaluation and community wide opt-out.
- Provide immediate relief to those requesting it and restore the analog meters.

Members of the Board
American Academy of Environmental Medicine

APPENDIX D American Academy of Pediatrics Statement

American Academy of Pediatrics
DEDICATED TO THE HEALTH OF ALL CHILDREN™

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July 12, 2012

The Honorable Julius Genachowski
Commissioner
Federal Communications Commission
445 12th Street SW
Washington, DC 20554

Dear Chairman Genachowski:

The American Academy of Pediatrics (AAP), a non-profit professional organization of 60,000 primary care pediatricians, pediatric medical sub-specialists, and pediatric surgical specialists dedicated to the health, safety and well-being of infants, children, adolescents, and young adults strongly supports the proposal for a formal inquiry into radiation standards for cell phones and other wireless products. The Academy encourages the Federal Communications Commission (FCC) to vote to move forward with this inquiry in an expeditious manner.

The FCC has not assessed the standard for cell phone radiation since 1996. According to industry groups, approximately 44 million people had mobile phones when the standard was set; today, there are more than 300 million mobile phones in use in the United States. While the prevalence of wireless phones and other devices has sky-rocketed, the behaviors around cell phone uses have changed as well. The number of mobile phone calls per day, the length of each cell phone call, and the amount of time people use mobile phones has increased, while cell phone and wireless technology has undergone substantial changes. Many more people, especially adolescents and young adults, now use cell phones as their only phone line and they begin using wireless phones at much younger ages.

The FCC standard for maximum radiation-exposure levels are based on the heat emitted by mobile phones. These guidelines specify exposure limits for hand-held wireless devices in terms of the Specific Absorption Rate (SAR), which measures the rate the body absorbs radiofrequency (RF). The current allowable SAR limit is 1.6 watts per kilogram (W/kg), as averaged over one gram of tissue. Although wireless devices sold in the United States must ensure that they do not exceed the maximum allowable SAR limit when operating at the device's highest possible power level, concerns have been raised that long-term RF exposure at this level affects the brain and other tissues and may be connected to types of brain cancer, including glioma and meningioma.

In the past few years, a number of American and international health and scientific bodies have contributed to the debate over cell phone radiation and its possible link to cancer. The International Agency for Research on Cancer (IARC), part of the

United Nations' World Health Organization, said in June 2011 that a family of frequencies that includes mobile-phone emissions is "possibly carcinogenic to humans." The National Cancer Institute has stated that although studies have not demonstrated that RF energy from cell phones definitively causes cancer, more research is needed because cell phone technology and cell phone use are changing rapidly. While a definitive link between cell phone radiation and brain cancer has not been established, these studies and others clearly demonstrate the need for further research into this area and highlight the importance of reassessing the current SAR to determine if it is protective of human health.

The AAP believes the inquiry to reassess the radiation standard presents an opportunity to review its impacts on children's health and well-being. In the past, such standards have generally been based on the impact of exposure on an adult male. Children, however, are not little adults and are disproportionately impacted by all environmental exposures, including cell phone radiation. In fact, according to IARC, when used by children, the average RF energy deposition is two times higher in the brain and 10 times higher in the bone marrow of the skull, compared with mobile phone use by adults. While the Academy appreciates that the FCC is considering investigating whether the emission standards should be different for devices primarily used by children, it is essential that any new standard for cell phones or other wireless devices be based on protecting the youngest and most vulnerable populations to ensure they are safeguarded throughout their lifetimes.

Finally, in reviewing the SAR standard, the FCC has the opportunity to highlight the importance of limiting media use among children. The Academy has found potentially negative effects and no known positive effects of media use by children under the age of two, including television, computers, cell phones, and other handheld wireless devices. In addition, studies consistently show that older children and adolescents utilize media at incredibly high rates, which potentially contributes to obesity and other health and developmental risks. In reviewing the SAR limit, the FCC has the opportunity to improve the health of our nation by highlighting the importance of limiting screen time and media use for children and adolescents.

The AAP supports the proposal for a formal inquiry into radiation standards for cell phones and other wireless products and the Academy encourages the FCC to vote in favor of moving forward with this investigation. If you have questions or concerns, please contact Kristen Mizzi in the AAP's Washington Office at 202/347-8600.

Sincerely,

Robert W. Block, MD FAAP President

Appendix E RCNIRP Resolution: Electromagnetic Fields from Mobile Phones: Health Effects on Children and Teenagers

"The Resolution evolved from scientific statements adopted by RNCNIRP in 2001, 2004, 2007, 2008 and 2009, taking into account contemporary views and actual scientific data. The Resolution represents a viewpoint of the professional scientific community and is meant for public dissemination, for the consumers of the mobile telecommunications services, as well as for the legislative and executive authorities who develop and implement health protection, environmental, communication, scientific and safety policies."

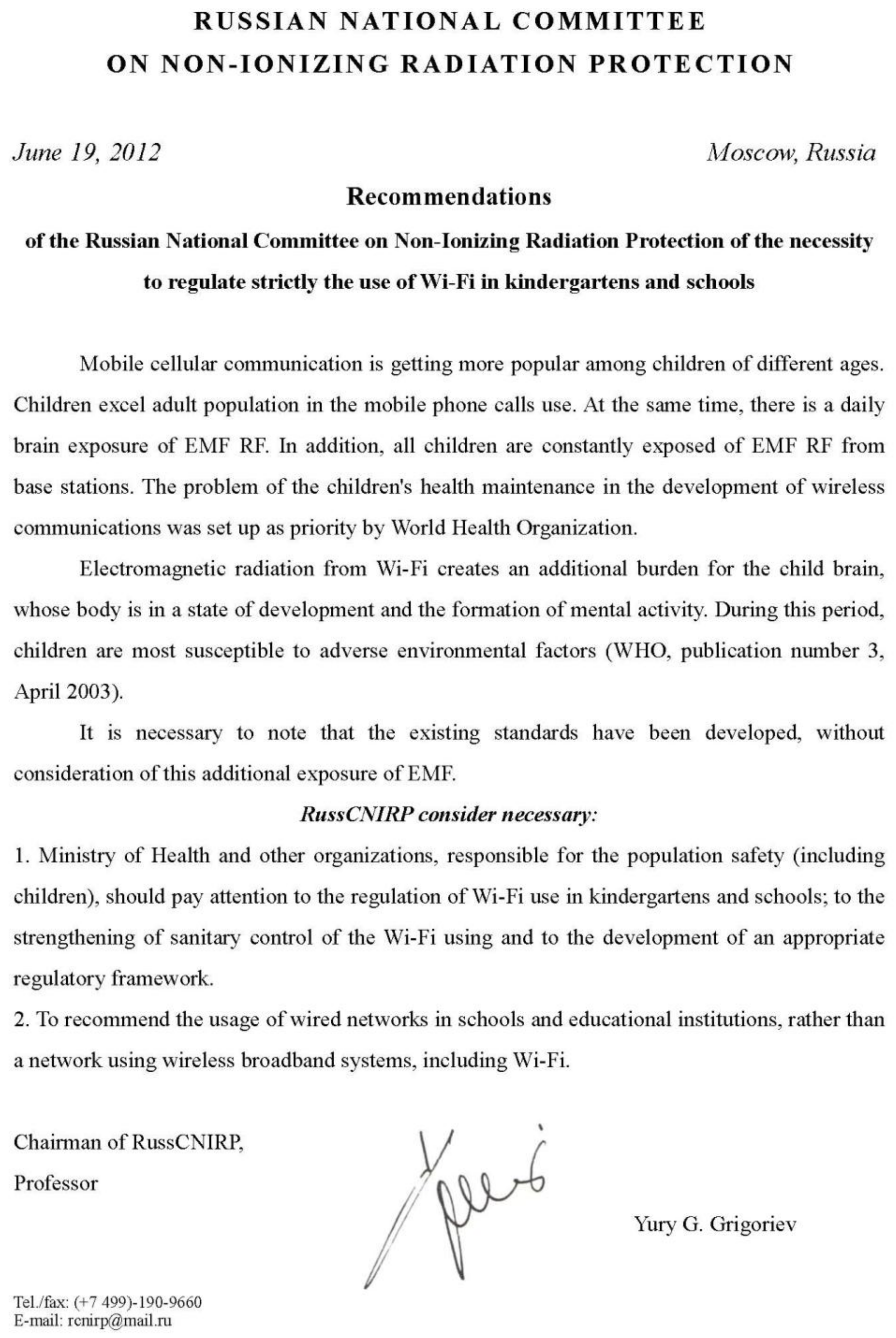
" ... Thus, for the first time in the human history, children using mobile telecommunications along with the adult population are included into the health risk group due to the RF EMF exposure. A situation has emerged that cumulative EMF exposure of children may be comparable to adult exposure and may be equal to the levels of occupational exposure of workers. At the same time, the society, with all its administrative and social structures, remain in a "waiting" position."

"Priority measures aimed at protection of children and teenagers

Taking into account the RNCNIRP position and the precautionary measures suggested by WHO, the Committee considers that urgent measures must be taken because of the inability of children to recognize the harm from the mobile phone use and that a mobile phone itself can be considered as an uncontrolled source of harmful exposure.

1. *It is required that the information that a mobile phone is a source of RF EMF is clearly shown on the phone's body (or any other telecommunication device).*
2. *It is required that the "User's Guide" contains information that a mobile phone (personal wireless communication tool using electromagnetic communication method, etc.) is a source of harmful RF EMF exposure. Usage of a mobile phone by children and adolescents under 18 years old is not recommended by the Sanitary Rule SanPiN 2.1.8/2.2.4.1190-03, and mobile phone use requires implementation of precautionary measures in order to prevent health risks. Mobile phone use by pregnant women is not recommended in order to prevent risk for a fetus.*
3. *The easiest way to reduce RF EMF exposure is to move the mobile phone away from one's head during the phone call which may be achieved by using the hands-free sets (protection by distance). Shortening the call duration is another way to reduce the exposure (protection by time).*
4. *The RNCNIRP considers it is reasonable to develop mobile phones with reduced EMF exposure (with hands-free sets, included limitation functions, such as limitation of the number of daily phone calls, possibility of forced limitation of phone call duration, etc.).*
5. *It is required to include courses on mobile phones use and issues concerning EMF exposure in the educational program in schools.*
6. *It is reasonable to set limits on mobile telecommunications use by children and adolescents, including ban on all types of advertisement of mobile telecommunications for children (teenagers) and with their participation.*
7. *The RNCNIRP is ready to assist the mass-media in their awareness-raising work and educational activities in the area of EMF and, in particular, to provide information about the newest research of the impact of EMF on human health and the measures to curb the negative impact of this physical agent.*
8. *Better safety criteria for children and teenagers are required in the nearest term. Features of the developing organism should be taken into account, as well as the significance of bioelectric processes for human life and activities, present and future conditions of EMF, prospects of technological and technical development should be addressed in a document of legal status.*
9. *Development of a funded national program for studying possible health effects from chronic EMF exposure of the developing brain is necessary."*

Appendix F RCNIRP Resolution to Regulate the Use of WI-FI in Schools



RUSSIAN NATIONAL COMMITTEE
ON NON-IONIZING RADIATION PROTECTION

June 19, 2012 *Moscow, Russia*

Recommendations

of the Russian National Committee on Non-Ionizing Radiation Protection of the necessity to regulate strictly the use of Wi-Fi in kindergartens and schools

Mobile cellular communication is getting more popular among children of different ages. Children excel adult population in the mobile phone calls use. At the same time, there is a daily brain exposure of EMF RF. In addition, all children are constantly exposed of EMF RF from base stations. The problem of the children's health maintenance in the development of wireless communications was set up as priority by World Health Organization.

Electromagnetic radiation from Wi-Fi creates an additional burden for the child brain, whose body is in a state of development and the formation of mental activity. During this period, children are most susceptible to adverse environmental factors (WHO, publication number 3, April 2003).

It is necessary to note that the existing standards have been developed, without consideration of this additional exposure of EMF.

RussCNIRP consider necessary:

1. Ministry of Health and other organizations, responsible for the population safety (including children), should pay attention to the regulation of Wi-Fi use in kindergartens and schools; to the strengthening of sanitary control of the Wi-Fi using and to the development of an appropriate regulatory framework.
2. To recommend the usage of wired networks in schools and educational institutions, rather than a network using wireless broadband systems, including Wi-Fi.

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Professor

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SECTION 23

The Precautionary Principle

“Late Lessons from Early Warnings: Towards Realism and Precaution with EMF?”

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Disclaimer.: The views expressed are those of the author and do not represent the views of the EEA or its Management Board. The author has no competing financial interest in the matters dealt with.

Prepared for the BioInitiative Working Group

2007

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I. INTRODUCTION

The histories of selected public and environmental hazards, from the first scientifically based early warnings about potential harm, to the subsequent precautionary and preventive measures, have been reviewed by the European Environment Agency.(“Late Lessons from Early Warnings: the Precautionary Principle 1896-2000”, EEA,2001). This paper summarises some of the definitional and interpretative issues that arise from the report and subsequent debates, such as the contingent nature of knowledge; the definitions of precaution, prevention, risk, uncertainty, and ignorance; the use of differential levels of proof; and the nature and main direction of the methodological and cultural biases within the environmental health sciences. These issues are relevant to EMF.

II. THE TWELVE “LATE LESSONS FROM EARLY WARNINGS

The paper does not address the specifics of EMF hazards, leaving it to the reader to apply, or not, the “Twelve late Lessons” that conclude the report. These lessons attempt to synthesise the fourteen historical experiences from the very different case study chapters into generic knowledge that can help inform policy-making on current issues such as GMO,, nanotechnologies, mobile phones, and endocrine disrupting substances where the luxuries of hindsight are not yet available but where exposures are already widespread and rising.

The idea of the twelve late lessons is to make the most of past experience to help anticipate future surprises whilst recognising that history never exactly repeats itself. When adopted alongside the best available science the lessons aim to help minimize hazards without compromising innovation. The “lessons” are reproduced below.

A. “Identify/Clarify the Framing and Assumptions”

1. Manage “risk”, “uncertainty” and “ignorance”
2. Identify/reduce “blind spots” in the science
3. Assess/account for all pros and cons of action/inaction
4. Analyse/evaluate alternative options

5. Take account of stakeholder values
6. Avoid "paralysis by analysis" by acting to reduce hazards via the precautionary principle.

B. "Broaden Assessment Information"

7. Identify/reduce interdisciplinary obstacles to learning
8. Identify/reduce institutional obstacles to learning
9. Use "lay", local as well as specialist knowledge
10. Identify/anticipate "real world" conditions
11. Ensure regulatory and informational independence
12. Use more long-term (ie. decades) monitoring and research

III. EARLY USE OF PRECAUTION

The Vorsorgeprinzip, or "foresight" principle, only emerged as a specific policy tool during the German debates on the possible role of air pollution as a cause of "forest death" in the 1970-80s. However, John Graham, one of Bush's science policy advisors, and trenchant critic of the precautionary principle, has noted that:

> *"Precaution, whether or not described as a formal principle, has served mankind well in the past and the history of public health instructs us to keep the spirit of precaution alive and well".* (Graham 2002).

Graham might have been thinking of the cholera episode of 1854 when precaution did indeed serve the people of London well. Dr. John Snow, a London physician, used the spirit of precaution to advise banning access to the polluted water of the Broad St. pump which he suspected was the cause of the cholera outbreak. He based his recommendation on the evidence he had been accumulating for some years including his study of S. London populations served by both piped and well water. Snow's views on cholera causation were not shared by The Royal College of Physicians who considered Snow's thesis and rejected it as 'untenable' as they and other "authorities" of the day believed that cholera was caused by airborne contamination. This particular scientific "certainty" soon turned out to be certainly mistaken, with the last remaining doubt being removed when Koch in Germany isolated the cholera vibrio in 1883.

From the *association* between exposure to water polluted with human faeces, and cholera, observed by Snow in 1854, to Koch's discovery of the *"mechanism of action"*, took 30 years of further scientific inquiry. Such a long time lag between acknowledging compelling associations and understanding their mechanisms of action is a common feature of scientific inquiry, as the histories of TBT, PCBs, DES, the Great Lakes pollution, beef hormones and the other cases in the EEA report illustrate.

IV. KNOWLEDGE AND IGNORANCE REQUIRES BOTH PREVENTION AND PRECAUTION

The Broad St. pump, TBT, DES, PCBs and Great Lakes Pollution examples described here also serve to illustrate the contingent nature of knowledge. Today's scientific certainties can be tomorrow's mistakes, and today's research can both reduce and increase scientific uncertainties, as the boundaries of the "known" and the unknown expand. Waiting for the results of more research before taking action to reduce threatening exposures may not only take decades but the new knowledge may identify previously unknown sources of both uncertainty and ignorance, as awareness of what we do not know expands, thereby supplying further reasons for inaction. "Paralysis by Analysis " can then follow.

"The more we know, the more we realise what we don't know" is not an uncommon scientific experience. Socrates observed some time ago:
"I am the wisest man alive, for I know one thing, and that is that I know nothing".
(Plato's Apology 1.21).

This was an early lesson in humility that has been lately forgotten by many scientists and politicians, who often put what turns out to be "misplaced certainty" in today's scientific knowledge: or assume that uncertainty can only be reduced, and not increased, by further research.

The distinction between uncertainty and ignorance is important. (Stirling, 1999) Ignorance is knowing that today's knowledge is very limited: it is the source of scientific surprises, such as the hole in the ozone layer, the mesothelioma cancer from asbestos, imposex in sea snails etc. It is distinct from the uncertainties that arise from gaps in knowledge and from variances in sampling and monitoring; parameter variability; model assumptions; and from the other attempts to approximate, model and predict unfolding realities.

Foreseeing and preventing hazards in the context of ignorance presents particular challenges to decision-makers. At first sight it looks impossible to do anything to avoid or mitigate "surprises". And ignorance ensures that there will always be surprises. However, some measures that could help limit the consequences of ignorance and the impacts of surprises are:

- using intrinsic properties as generic predictors for unknown but possible impacts e.g. the persistence, bioaccumulation and spatial range potential of chemical substances. (Stroebe et al., 2004)
- reducing specific exposures to potentially harmful agents on the basis of credible 'early warnings' of *initial* harmful impacts, thus limiting the size of any other 'surprise' impacts from the same agent, such as the asbestos cancers that followed asbestosis; and the PCB neurotoxicological effects that followed its wildlife impacts.
- promoting a diversity of robust and adaptable technological and social options to meet needs, which limits technological 'monopolies' (such as those like asbestos, CFCs, PCBs etc.), and therefore reduces the scale of any 'surprise' from any one technological option.
- using more long-term research and monitoring of what appear to be "surprise sensitive sentinels", such as frogs and foetuses.

A. Prevention and Precaution

The distinction between *prevention* and *precaution* is also important. Preventing hazards from "known" risks is relatively easy and does not require precaution. Banning smoking, or asbestos, today requires only acts of prevention to avoid the well-known risks. However, it would have needed precaution, (or foresight, based on a sufficiency of evidence), to have justified acts to avoid exposure to the then uncertain hazards of asbestos in the 1930s –50s, or of tobacco smoke in the 1960's). Such precautionary acts then, if implemented successfully, would have saved many more lives in Europe than today's bans on asbestos and smoking are doing. As

Cogliano has recently pointed out, the difference between prevention and precaution can be further illustrated by showing that *prevention* is used to justify the restriction of exposure to an IARC Category 1 carcinogen whereas *precaution* is necessary to justify restricting exposure to a Category 2A or B carcinogen, where the evidence is less strong. The section below. on different levels of proof , further elaborates this point.

For EMF, the question is, does the existing strength of evidence justify *precautionary* actions now? Or will exposure reduction be delayed until the evidence is clear enough to justify the more belated and overall less protective *prevention* of “known” causes, so that EMF replicates the fate of asbestos ,smoking and most of the other cases in the EEA report?

Some commentators, who have a long and distinguished history in preventing occupational and environmental risks, have queried the added value of the precautionary principle in the field of public health, with its long traditions of prevention. (Goldstein, 2007).

The key to understanding the added value of the PP requires a) acknowledging the distinction between prevention and precaution described above; b) an appreciation of the further distinctions between the primary, secondary and tertiary preventative *measures* that have long between adopted in public health, and the prior *justification* for any such measure, which the PP brings; and c) a recognition of the increased legitimacy and transparency that arises from the articulation and adoption of the PP in legal texts, international agreements and conventions, as opposed to being merely part of general practice.

More empirically, the evidence that many scientific disciples, legal scholars (de Sadeleer, 2007),and international policymakers, have, since the 1970s, recognised the need for legitimising the PP as a new policy tool that is better able to deal with systems complexities, ignorance and uncertainties, suggests that the PP brings added value to the protection of the environment and the public.

There is much discussion generated by the different meanings often attached to the common terms “prevention”, “precaution”, “risk”, “uncertainty” and “ignorance”. **Table 1** attempts to clarify these so as to help reduce unnecessary argumentation.

Table 1: Clarification of Key Terms

Situation	***State and dates of knowledge***	***“Nature of the justification for Action”***
Risk	**‘Known’ impacts; ‘known’ probabilities e.g. asbestos**	**Prevention: action taken to reduce known hazards e.g. eliminate exposure to asbestos dust**
Uncertainty	**‘Known’ impacts; ‘unknown’ probabilities e.g. antibiotics in animal feed and associated human resistance to those antibiotics**	**Precautionary prevention: action taken to reduce exposure to potential hazards**
Ignorance	**‘Unknown’ impacts and therefore ‘unknown’ probabilities eg the ‘surprises’ of chlorofluorocarbons (CFCs), pre 1974**	**Precaution: action taken to anticipate, identify and reduce the impact of ‘surprises’**

Source: Reproduced, with amendment, from the Late Lessons Report, EEA 2001.

V. THE PRECAUTIONARY PRINCIPLE: DEFINITIONS AND INTERRPRETATIONS

There are some relatively rare but successful acts of “precautionary prevention” in the EEA report such as on cholera in1854, on TBT in France in the 1980s, and on CFCs in the 1970s. Together with the many other examples of the failure to use the precautionary principle in the other case studies (EEA, 2001), these illustrate the wisdom of taking appropriate precautionary actions to avoid plausible and serious threats to health or environments, especially when the impacts are irreversible and likely to be much more costly to society than the precautionary measures.

Some commentators have stressed the need for policymakers to take account of the foreseeable, or plausible, countervailing (secondary) costs of otherwise genuine precautionary attempts to protect the environment and health. (Rushton, 2007). This consideration of countervailing costs has long been recognised by the better policymakers, even if it is difficult in practice to anticipate and account for all consequences of actions. And of course there are the secondary benefits of precautionary actions as well, which tend to be less stressed, such as the benefit of reduced respiratory and cardiovascular disease from the reduced combustion of fossil fuels: a large and early secondary benefit of that climate change measure.

The outcomes of some controversial actions based on the PP, such as the EU ban on antibiotics as growth promoters, which is a Late Lessons case study, have since been scrutinised, and have been considered sound ,or unsound, depending on the science used and its interpretation by different interests. (Cox, 2007, Angulo et al., 2004).

Any policy effectiveness analysis of measures taken to deal with such multi-causal and long term hazards as antibiotics as growth promoters is fraught with methodological difficulties and is hampered by long latencies and complex biological systems: untangling the causal impact of one stressor amongst many inter-dependent ones is virtually impossible. The value of applying more probabilistic and value of information data to such conundrums is recognised by many risk managers. However, this cannot remove the need for scientific and political judgment about how to take appropriate and proportionate action in the face of irreducible uncertainties, ignorance and plausible hazards which could have serious, widespread and irreversible impacts and for which probabilities are not possible at the time when they are most needed. This is the current case with many EMF exposures.

A. Some Definitions and Interpretations of the Precautionary Principle

The increasing awareness of complexity and uncertainty during the 1980/90’s led to the German debates on the Vorsorgeprinzip shifting to the international level, initially in the field of conservation (World Charter for Nature UN 1982), but then particularly in marine pollution, where an overload of data accompanied an insufficiency of knowledge. (Marine Pollution Bulletin, 1997). This generated the need to act with precaution to reduce the large amounts of chemical pollution entering the North Sea. Since then many international treaties have included the PP (including the often cited version from the Third North Sea Ministerial Conference, 1990, have included reference to the precautionary principle, or, as they refer to it in the USA, the precautionary approach.

The N.Sea declaration called for *"action to avoid potentially damaging impacts of substances, even where there is no scientific evidence to prove a causal link between emissions and effects"*.

This definition has often, and sometimes mischievously, been used to deride the precautionary principle by claims that it appears to justify action even when there is "no scientific evidence" that associates exposures with effects. However, the N. Sea Conference definition clearly links the words "no scientific evidence" with the words. "to prove a causal link". We have already seen with the Broad St. pump and TBT examples that there is a significant difference between evidence about an "association" and evidence that is robust enough to establish a "causal" link. (Hill, 1965).

The Treaty of the European Union also cites the precautionary principle, as well as the other key principles of sound public policy on health:

> "*Community policy on the environment … shall be based on the precautionary principle and on the principles that preventive action should be taken, that environmental damage should, as a priority, be rectified at the source and the polluter should pay*" (Treaty on European Union, 1992).

Other parts of the EU Treaty ,and cases taken at the European Court of Justice, make it clear that these principles also apply to environmental and consumer protection issues.

These principles, as well as the important and legally required *proportionality principle,* which limits disproportion between the costs and benefits of prevention, are not defined in the Treaty but are illuminated by their practical application in case law. However, all serious applications of the precautionary principle require some scientific evidence of a plausible association between exposures and current, or potential, impacts.

There is still much disagreement and discussion about the interpretation and practical application of the precautionary principle, due, in part, to this lack of clarity and consistency over its definition. For example, many definitions in the Treaties and Conventions use a double negative to define the precautionary principle: that is, they identify reasons that cannot be used to justify not acting, but without specifying that a sufficiency of evidence is needed to justify taking action.

B. Reasonable Grounds for Concern?

The Communication from the EU on the precautionary principle (European Commission 2000) does specify that "reasonable grounds for concern" are needed to justify action under the precautionary principle, but it does not make explicit that these grounds will be case specific: nor does it explicitly distinguish between risk, uncertainty and ignorance. Since the EC Communication, the EU Council of Misisters, EU case law, and the regulation establishing the new European Food Safety Authority, EFSA, (General Food Law Regulation, EC No 178/2002), have further clarified the circumstances of use and application of the precautionary principle.
For example, the judgement of the European Court of Justice in the BSE case contained a general definition which authoritative commentators think contain many of the necessary elements of the precautionary principle that are applicable in all areas of the EC law:

> "*Where there is uncertainty as to the existence or extent of risks to human health, the institutions may take protective measures without having to wait until the reality and seriousness of those risks become fully apparent*" (Christoforou, 2002).

The WHO Declaration from the Fourth Ministerial Conference on Environment and Health (WHO, 2004a) refers explicitly to the precautionary principle with the recommendation:

> *"that it should be applied where the possibility of serious or irreversible damage to health or the environment has been identified and where scientific evaluation, based on available data, proves inconclusive for assessing the existence of risk and its level but is deemed to be sufficient to warrant passing from inactivity to policy alternatives"* (WHO, 2004b).

The American Public Health Association (APHA) affirmed endorsement of the precautionary principle as a cornerstone of public health for the protection of children's health. In a 2000 policy statement, the APHA encouraged governments, the private sector and health professionals to promote and use the precautionary principle to protect the health of developing children (APHA, 2001).

C. The EEA working definition of the Precautionary Principle.

The working definition used in the European Environment Agency that has been developed during debates since 2001 is explicit about specifying both uncertainty and ignorance, as contexts for applying the principle, and in acknowledging that a case-specific sufficiency of scientific evidence is needed to justify public policy actions:

> '*The Precautionary Principle provides justification for public policy actions in situations of scientific complexity, uncertainty and ignorance, where there may be a need to act in order to avoid, or reduce, potentially serious or irreversible threats to health or the environment, using an appropriate level of scientific evidence, and taking into account the likely pros and cons of action and inaction*' (Gee, 2006).

The definition is also explicit about the trade off between action and inaction, and widens the conventionally narrow, and usually quantifiable, interpretation of costs and benefits to embrace the wider and sometimes unquantifiable, "pros and cons". Some of these wider issues, such as loss of the ozone layer, or of public trust in science, are unquantifiable, but they can sometimes be more damaging to society than the quantifiable impacts: and they need to be included in any comprehensive risk assessment. The EEA definition is proving to be useful in clarifying the use and interpretation of the PP, especially in emerging issues such as EMF.

VI. DIFFERENT LEVELS OF PROOF FOR DIFFERENT PURPOSES

The level of proof (or strength of scientific evidence) that would be appropriate to justify public action in each case varies with the pros and cons of action or inaction. These include the nature and distribution of potential harm; the justification for, and the benefits of the agent or activity under suspicion; the availability of feasible alternatives; and the overall goals of public policy. Such policy goals can include the achievement of the "high levels of protection" of public health, of consumer safety, and of the environment, required by the EU Treaty.

The use of different levels of proof is not a new idea: societies often use different levels of proof like for different purposes.

For example, a high level of proof (or strength of evidence) such as “beyond all reasonable doubt” is used to achieve good science where A is seen to cause B only when the evidence is very strong. Such a high level of proof is also used to minimise the costs of being wrong in the criminal trial of a suspected murderer, where it is usually regarded as better to let several guilty men go free than it is to wrongly convict an innocent man. However, in a different, civil trial setting, where, say, a citizen seeks compensation for neglectful treatment at work, which has resulted in an accident or ill health, the court often uses a lower level of proof commensurate with the costs of being wrong in this different situation. I n compensation cases an already injured party is usually given the benefit of the doubt by the use of a medium level of proof, such as “balance of evidence or probability”. It is seen as being less damaging (or less costly in the wider sense) to give compensation to someone who was *not* treated negligently than it is to *not* provide compensation to someone who was treated negligently. The “broad shoulders” of insurance companies are seen as able to bear the costs of mistaken judgements rather better than the much narrower shoulders of an injured citizen. In each of these two illustrations it is the nature and distribution of the costs of being wrong that determines the level of proof (or strength of evidence) that is “appropriate” to the particular case.

Bradford Hill, cited above, was very concerned about the social responsibility of scientists and he concluded his classic 1965 paper on association and causation in environmental health , which was prepared at the height of the smoking controversy, with a “call for action” in which, *inter alia*, he also proposed the concept of case specific and differential levels of proof. His three examples ranged from “relatively slight” to “very strong” evidence, depending on the nature of the potential impacts and of the pros and cons in each specific case, i.e., possibly teratogenic medicine for pregnant women; a probable carcinogen in the workplace; and government restrictions on public smoking or diets. (Bradford Hill 1965).

Identifying an appropriate level of proof has also been an important issue in the climate change debates. The International Panel on Climate Change (IPCC) discussed at length this issue before formulating their 1995 conclusion that “on the balance of evidence” mankind is disturbing the global climate. They further elaborated on this issue in their 2001 report where they identified 7 levels of proof (or strengths of evidence) that can be used to characterise the scientific evidence for a particular climate change hypothesis.

Table 2 provides the middle 5 of these levels of proof from the IPPC and illustrates their practical application to a variety of different societal purposes. In the cancer field the International Agency for Research on Cancer also uses several strengths of evidence to characterise the scientific evidence on carcinogens. (Cogliano, 2007)

Different Levels of Proof for Different Purposes: Some Examples and Illustrations

Probability	Quantitative descriptor (Probability bands based on IPCC 2001)	Qualitative Descriptor	Illustrations
100 % probability	Very likely (90-99 %)	• “Statistical significance”	• Part of strong scientific evidence for “causation”
90 %		• “Beyond all reasonable doubt”	• Most criminal law. And the Swedish Chemical law, 1973, for evidence of ”safety” of substances under suspicion- burden of proof on manufacturers
	Likely (66-90 %)	• “Reasonable certainty”	• Food Quality Protection Act, 1996 (US)
		• “Sufficient scientific evidence”	• To justify a trade restriction designed to protect human, animal or plant health under World Trade Organisation Sanitary and Phytosanitary (SPS) Agreement, Art. 2.2, 1995
50 %	Medium Likelihood (33-66 %)	• ”Balance of evidence”	• Intergovernmental Panel on Climate Change 1995 & 2001
		• ”Balance of probabilities”	• Much Civil and some administrative law
		• ”Reasonable grounds for concern”	• European Commission Communication on the Precautionary Principle 2000
		• ”Strong possibility”	• British Nuclear Fuels occupational radiation compensation scheme, 1984 (20-50% probabilities triggering different awards up to 50% +, which then triggers full compensation)
	Low Likelihood (10-33 %)	• ”Scientific suspicion of risk”	• Swedish Chemical law, 1973, for sufficient evidence to take precautionary action on potential harm from substances-burden of proof on regulators
0 %		• ”Available pertinent information”	• To justify a provisional trade restriction under WTO SPS Agreement, Art. 5.7 where ”scientific information is insuffiicient”
	Very Unlikely (1-10 %)	• Low risk	• Household fire insurance
0 % probability		• ”Negligible and insignificant”	• Food Quality Protection Act, 1996 (US)

Source: EEA, 2001

VII. FALSE NEGATIVES AND FALSE POSITIVES.

All of the 14 case studies (tributylin or TBT, benzene, PCBs, CFCs, MTBE, SO_2. Great Lakes pollution. DES, and beef hormones. asbestos. medical x-rays, BSE and Fisheries are all examples of "false negatives" in the sense that the agents or activities were regarded as not harmful for some time before evidence showed that they were indeed hazardous.

We tried to include a "false positive" case study in the report (i.e., where actions to reduce potential hazards turned out to be unnecessary), but failed to find either authors or sufficiently robust examples to use. Providing evidence of "false positives" is more difficult than with "false negatives" (Mazur, 2004). How robust, and over what periods of time, does the evidence on the absence of harm have to be before concluding that a restricted substance or activity is without significant risk?

Volume 2 of "Late Lessons", which the EEA intends to publish in 2008, will explore the issues raised by false positives. including lessons to be learned from such apparent examples as the EU ban on food irradiation and hazardous labelling on saccharin in the US. The Y2K computer bug story may also carry some interesting lessons.

Why are there so many "false negatives" to write about, and how might this be relevant to EMF? Conclusions based on the first Late lessons volume of case studies point to two main answers: the bias within the health and environmental sciences towards avoiding "false positives", thereby generating more "false negatives", and the dominance within decision-making of short-term, specific, economic and political interests over the longer term, diffuse, and overall welfare interests of society.

The latter point needs to be further explored, particularly within the political sciences. Researchers could examine the ways in which society's long-term interests can be more effectively located within political and institutional arrangements that have, or could have, an explicit mandate to look after the longer term welfare of society, and thereby to better resist the short-term pressures of particular economic or political interests. The judiciary in democracies can play part of this role, as can long running and independent advisory bodies, such as the Royal Commission on Environmental Pollution (UK), or the German Advisory Council on Global Change.

The current and increasing dominance of the short-term in markets and in parliamentary democracies makes this an important issue. The experiments we are conducting with planet earth, its eco-systems and the health of its species, including humans, require, *inter alia*, more long-term monitoring of “surprise-sensitive” parameters which could, hopefully, give us early warnings of impending harm.
Such long-term monitoring requires long-term funding, via appropriately designed institutions: such funding and institutions are in short supply. The case studies in Vol. 1 of “Late Lessons” illustrate both the great value, (e.g. in the TBT, DES, Great Lakes and CFCs stories), yet relative paucity, of long-term monitoring of both health and environments. Such monitoring can contribute to the “patient science” that slowly evolving natural systems require for their better understanding.

Since the publication of “Late Lessons” we have further explored the second cause of “false negatives” i.e. the issue of bias within the health and environmental sciences. Table 3 lists sixteen common features of methods and culture in the environmental and health sciences and shows their main directions of error. Of these, only three features tend towards generating “false positives” whereas twelve tend towards generating “false negatives”. (Clearly, the weighting of these different biases would be the next step but has not yet been tried).

Table 3

ON BEING WRONG:
Environmental and Health Sciences and Their Directions of Error

SCIENTIFIC STUDIES	**SOME METHODOLOGICAL FEATURES**	**MAIN[1] DIRECTIONS OF ERROR-INCREASES CHANCES OF DETECTING A:**
Experimental Studies (Animal Laboratory)	• High doses	• False positive
	• Short (in biological terms) range of doses	• False negative
	• Low genetic variability	• False negative
	• Few exposures to mixtures	• False negative
	• Few Foetal-lifetime exposures	• False negative
	• High fertility strains	• False negative (Developmental/reproductive endpoints)
Observational Studies (Wildlife & Humans)	• Confounders	• False positive
	• Inappropriate controls	• False positive/negative
	• Non-differential exposure misclassification	• False negative
	• Inadequate follow-up	• False negative
	• Lost cases	• False negative
	• Simple models that do not reflect complexity	• False negative
Both Experimental And Observational Studies	• Publication bias towards positives	• False positive
	• Scientific cultural pressure to avoid false positives	• False negative
	• Low statistical power (e.g. From small studies)	• False negative
	• Use of 5 % probability level to minimise chances of false positives	• False negative

Source: Gee, 2006

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[1] Some features can go either way (e.g. inappropriate controls) but most of the features mainly err in the direction shown in the table

The general bias towards the null helps to produce robust science, basing it on strong foundations of knowledge, but this bias can encourage poor public health or environmental policy. The goals of science and public policy-making on health and environmental hazards are different: science puts a greater priority on avoiding "false positives" by accepting only very high levels of proof of "causality", whereas public policy tries to prioritize the avoidance of "false negatives" on the basis of a sufficiency of evidence of potential harm.

Table 3 is derived from papers presented to a conference on the precautionary principle organised by the Collegium Ramazzini, the EEA, the WHO and NIEHS in 2002. (Grandjean et al., 2003). It provides a first and tentative step in trying to capture and communicate the main directions of this bias within the environmental and health sciences, a bias which decision makers and the public should be aware of. As they debate the evidence on emerging hazards such as EMF.

The appropriate balance between false negatives and positives was addressed at a JRC/EEA workshop on the precautionary principle and scientific uncertainty which was held during the "Bridging the Gap" Conference, 2001, organised by the Swedish Presidency of the EU, in partnership with the EEA and DG Research. It drew the following conclusion:

> *"Improved scientific methods to achieve a more ethically acceptable and economically efficient balance between the generation of "false negatives" and "false positives" are needed".* (Swedish EPA 2001).

VIII. SOME CRITERIA FOR ESTABLISHING CAUSATION

Bradford Hill established nine criteria for helping to move from association to causation in environmental health which have been, and still are, widely used in debates on issues such as EMF

Two of the apparently more robust of the "criteria" may not be so robust in the context of multi-causality, complexity and gene/host variability.

For example, ***"consistency"*** of study findings is not always to be expected. As Prof. Needleman, who provided the first of what could be called the second generation of early warnings on lead in petrol in 1979 has observed:

> *"Consistency in nature does not require that all or even a majority of studies find the same effect. If all studies of lead showed the same relationship between variables, one would be startled, perhaps justifiably suspicious"* (Needlemann , 1995).

It follows that the *presence* of consistency of results between studies on the same hazard can provide robust evidence for a causal link, but the *absence* of such consistency may not provide very robust evidence for the absence of a real association. In other words, the "criterion" of consistency is asymmetrical, like most of the other Bradford Hill "criteria".

Similarly, the criterion of ***"temporality",*** which says that the putative cause X of harm Y must come before Y appears, is robust in a simple, uni-causal world. In a multi-causal, complex world of common biological end points that have several chains of causation this may not necessarily be so. For example, falling sperm counts can have multiple, co-causal factors, some of which may have been effective at increasing the incidence of the biological end point in question in advance of the stressors in focus, thereby confusing the analysis of temporality. The resulting overall sperm count trends could then be rising, falling or static, depending on the combined direction and strengths of the co-causal factors and the time lags of their impacts. It follows that say, chlorine chemicals, may or may not be co-causal factors in falling sperm counts: but the use of the "temporality" argument by the WHO, who observed that sperm counts began to fall before chlorine chemistry production took off, does not provide robust evidence that they are not causally involved.

The presence of "temporality", like "consistency" may be robust evidence *for* an association being causal, but its *absence* may not provide robust evidence *against* an association. Bradford Hill was explicitly aware of the asymmetrical nature of his "criteria": his followers have not always been so aware.
During 2005, the 40th anniversary year of the Bradford Hill "criteria", the EEA convened a panel of experts to review the "criteria" and their use in light of advances in knowledge, particularly multi-causality, since 1965. A report will be published in 2007.

How this goal can be achieved without compromising science remains to be explored, (Grandjean 2004; Grandjean et al., 2004). It is clearly necessary, particularly when dealing with EMF, for scientific methods to not only take account of thisfalse negative/positive bias in methodologies but also to more clearly reflect other realities such as multi-causality; thresholds; timing of dose; sensitive sub-populations, such as children, (Jarosinska and Gee, 2007); sex, age, and immune conditions of the host; information physics; effects below the thresholds of "acute" impacts, such as tissue heating; non-linear dose/response relationships; "low dose" effects; and the effects arising from disturbing the balance between opposing elements in complex biological systems. The evidence on EMF needs to take full account of these realities, as well as of the methodological biases of Table 3.

1X. PUBLIC PARTICIPATION IN RISK ANALYSIS

Choosing an appropriate level of proof for a particular case is clearly based, *inter alia,* on value judgements about the acceptability of the costs, and of their distribution, of being wrong in both directions, i.e. of acting or not acting to reduce threatening exposures. This is why it is necessary to involve the public in decisions about serious hazards and their avoidance: and to do so for all stages of the risk analysis process.

Three of the "twelve late lessons" (number 5, number 9 and number 10) explicitly invite early involvement of the public and other stakeholders at all stages of risk analysis, a development which has been actively encouraged in many other influential reports during the last decade. (NRC 1994; US Presidential Commission on Risk Assessment and Risk Management 1997; Royal Commission on Environmental Pollution 1998; CEC Communication on the Precautionary Principle 2000; German Advisory Council on Global Change 2001).

The best available science is therefore only a necessary but not a sufficient condition for sound public policy making on potential threats to health and the environment. Where there is scientific uncertainty and ignorance "it is primarily the task of the risk managers to provide risk assessors with guidance on the science policy to apply in their risk assessments." (Christoforou, 2003). The content of this science policy advice, as well as the nature and scope of the questions to be addressed by the risk assessors, need to be formulated by the risk managers and relevant stakeholders at the initial stages of the risk analysis.

Involving the public in not only all stages of risk analysis, but also in helping to set research agendas and technological trajectories, (Wilsdon and Willis, 2004) is not easy. Many experiments, in both Europe and the USA, with focus groups, deliberative polling, citizens' juries, and extended peer review, (Funtovicz and Ravetz, 1990/92) are exploring appropriate ways forward.

The issue of time is also a critical issue for risk analysis and application of the precautionary principle. For example, the time from the first scientifically based early warnings (1896 for medical X rays, 1897 for benzene, 1898 for asbestos) to the time of policy action that effectively reduced damage was often 30-100 years. Some consequences of the failures to act in good time (e.g. on CFCs or asbestos) continue to cause damage over even longer time periods. For example, the ozone hole will cause many thousands of extra skin cancers in today's children but the cancers will only peak around the middle of this century because of the long latent period between exposure and effect. Such long-term but foreseeable impacts raise liability and compensation issues, including appropriate discount rates (if any) on future costs and benefits, which being value-laden choices. need also to be discussed by stakeholder groups. Again, experience in the climate change field with these long-term issues may be helpful in managing them with respect to electromagnetic fields (ELF and RF).

The wider involvement of stakeholders has also been recognised more recently by the International Risk Governance Council (IRGC, 2005) and the EU report on Science and Governance, (Wynne et al., 2007). Whether wider involvement of stakeholders results in better and more acceptable decisions needs to studied: early indications (Beierle, 2002), and lessons from history, suggests that is. In many cases several decades will be necessary to confidently judge outcomes, given latencies and complexities.

X. SOME EXAMPLES OF EARLY WARNINGS

The 14 case studies in the Late Lessons Report (EEA 2001) include examples some chemicals (tributylin or TBT, benzene, PCBs, CFCs, MTBE, SO_2 and Great Lakes pollution); two other pharmaceuticals (DES, and beef hormones); two physical agents (asbestos and medical x-rays); one pathogen (BSE); and Fisheries (overfishing).

The main issues discussed so far, such as the contingent nature of knowledge; ignorance and "surprises"; appropriate levels of evidence for policy actions; and public participation in risk analysis are critical to the successful application of both scientific knowledge and the precautionary principle to public policy-making. They are therefore relevant to discussions about the potentially new hazards that are now emerging e.g. from nanotechnology, (Royal Society 2003); from the non-ionising radiations arising from the use of mobile phones, (Stewart Reports 2000, 2004), and from endocrine disrupting substances or EDSs. (WHO, 2002).

With such newly emerging hazards it can be helpful to use historical examples to illustrate what a scientifically based early warning looks like as it is often difficult to properly recognise such warnings at the time they occur. A good example is that provided by the UK Medical Research Council's Swann Committee in 1969. They were asked to assess the evidence for risks of resistance to antibiotics in humans following the prolonged ingestion of trace amounts of antibiotics arising from their use as growth promoters in animal feed. (Edqvist and Pedersen 2001). They concluded that:

> *"Despite the gaps in our knowledge .. we believe … on the basis of evidence presented to us, that this assessment is a sufficiently sound basis for action .. The cry for more research should not be allowed to hold up our recommendations'….'sales/use of AFA should be strictly controlled via tight criteria, despite not knowing mechanisms of action, nor foreseeing all effect".* (Swann 1969).

A. Antibiotics in Animal Feed

The Swann Committee also concluded that it would be more rewarding and innovative to improve animal husbandry as a means of encouraging disease free animal growth rather than to the cruder approach of diets containing antimicrobials. Despite the gaps in knowledge, the need for much more research, and considerable ignorance about the mechanisms of action, a sufficiency of evidence was identified and described by the Swann Report that justified the need for public authorities to restrict the possibility of exposures to antibiotics from animal growth promoters. This early warning was initially heeded, but was then progressively ignored by the pharmaceutical companies and regulatory authorities, who wanted more scientific justification for restricting anti-microbial growth promoters. However, in 1985 in Sweden, and then in the EU in 1999, the use of antibiotics as growth promoters was finally banned. Pfizer, the main supplier of such antibiotics in Europe, appealed against the European Commission banning decision, pleading, *inter alia*, an insufficiency of scientific evidence. They lost this case at the European Court of Justice (Case T-13/99-Pfizer 2002), a case which further clarified the proper use and application of the precautionary principle in circumstances of scientific uncertainty and of widespread, if low, public exposures to a potentially serious threat.

B. Lead in Gasoline

A US example of an early warning comes from the lead in gasoline story: a warning that was largely ignored for over 50 years, resulting in much damage to the intelligence and behaviour of children in America, Europe and the rest of the motorised world. Yandell Hendersson, Chair of the Medical Research Board, US Aviation Service, who had been asked to look at the scientific evidence on the possible hazards of tetraethyl lead during the temporary ban on lead in petrol, in 1925, concluded:

> *"It seems likely that the development of lead poisoning will come on so insidiously that leaded gasoline will be in nearly universal use … before the public and the government awakens to the situation".* (Rosner and Markowitz, 2002).

Motorised societies would have gained much in dollars, brainpower and social cohesion had they heeded this foresight.

C. Tributylin (TBR) – A Marine Antifoulant for Ships

The case study on tributylin (TBT) and DES illustrate the surprises that arise from real life complexities and which may carry some lessons for the EMF debate. For example, the unfolding of the TBT story was accompanied by an increased appreciation of scientific complexity arising from the discoveries that adverse impacts were caused by very low doses (i.e. in parts/trillion); that high exposure concentrations were found in unexpected places e.g. in the marine micro-layer; and that bioaccumulation in higher marine animals, including sea-food for human consumption, was greater than expected. The early actions on exposure reduction in France and the UK in 1982-85 were based on a 'strength of evidence' for the 'association' only: knowledge about 'causality', **'**mechanisms of action' and other the complexities above came much later.

We were lucky in some ways with the TBT story: a highly specific, initially uncommon impact (imposex) was quickly linked to one chemical, TBT. This relatively easily identified linkage is not likely to be repeated for the more common and multi-causal impacts where, for example, neurodevelopmental diseases and dysfuntions, or common cancers, are the impacts under suspicion.

D. Diethylstilbestrol (DES)

Key lessons from the DES story are also instructive, as it provides the clearest example of endocrine disruption in humans. Diethylstilbestrol, commonly referred to as DES, is a synthetic estrogen . It was originally prescribed to prevent miscarriage, but did not. Later, sons and daughters of mothers given DES to prevent miscarriage developed cancers, reproductive tract anomalies, and had more pre-term babies themselves as a result. The effects of DES include the absence of visible and immediate teratogenic effects **not** being robust evidence for the absence of reproductive toxicity; and the **'**timing of the dose clearly determining the poison', in contrast to the 'dose determines the poison' dictum of Paracelsus. Timing is also relevant to other biological end points:

> *"the time of life when exposures take place may be critical in defining dose-response relationships of EDSs for breast cancer as well as for other health effects",*
> (WHO/IPCS, 2002).

Although the exposure levels were higher than the usual environmental levels of other EDSs, the DES story provides a clear warning about the potential dangers of perturbing the endocrine system with synthetic chemicals.

With over 20,000 publications, DES is now a well-studied compound, yet many doubts persist about its mechanisms of action. Since no dose-effect relationship has been found in humans, it cannot be excluded that DES could have been toxic at low doses, and that other less potent xenoestrogens could have similar effects.

If we still have few certainties about DES after so much time and research, what should our attitude be towards emerging hazards, such as other endocrine disrupting substances (EDSs) and EMF?

XI. CONCLUSION

The lessons of history from the EEA report, and subsequent debates and events, indicate that they have much relevance to the EMF issue, as well as to other emerging issues such as nanotechnology, (Royal Society, 2003) and endocrine disrupting substances or EDSs (WHO, 2002). The public health assessment of EMF could apply these lessons, approaches, terms of discussion and interpretations to the precautionary and preventative actions on the different parts of the EMF exposure problem.

There are of course large differences between smoking and EMF. The smoking hazard had at least 10 times the relative risk increase in the exposed population compared to the leukaemia risk from power line exposure; and the size of the smoking exposed population, and its exposure above that needed to generate a doubling of the risk, are both very much greater than with power lines. The larger relative risk for smoking and lung cancer seems to arise from comparing smokers with non, or never, smokers whilst the relative risk of 2 to 3 that arises between moderate and heavy smokers, or between second hand smokers and non smokers, is more relevant to the EMF issue, where there is an absence of unexposed controls. The lower relative risks of 2 or 3 for EMF are biased towards the null to unknown extent by the absence of such controls (Milham, 1998). However, the parallel between the slow recognition of the smoking hazard and power line EMF hazard is interesting.

The parallel with the history of X rays is also pertinent. The initial discovery, by Alice Stewart in the early 50s, that a few x rays of a pregnant woman in the sensitive period of her pregnancy gave a 2 fold excess of leukaemia, was greeted with much strident disbelief, particularly from the male doctors whose latest toy was under threat. It took another 20 years or so before her result became generally accepted, and only after several negative studies that were conducted in the early response to her study. Many studies of X rays in pregnant women now exist, and, as with the power line studies, the relative risk remains at about 2. (EEA, 2001) What will the history of EMF look like in 2020?

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Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

SECTION 23

The Precautionary Principle

2012 Supplement

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I. INTRODUCTION

In 2007, the evidence for EMF, and in particular radiofrequency radiation (RFR) from the use of mobile phones, was a focus for discussion in the BioInitiative Report (2007). It arose from growing scientific evidence of possible health risks, with a very large global population that could presumably be affected by the outcome.

Illustrating the importance of observing 'early warnings' of environmental and public health risks arising from emerging scientific studies and direct observation of impacts to peoples' health, this author wrote about the importance of applying 'lessons learned' from the histories of selected public and environmental hazards, from the first scientifically based early warnings about potential harm, to the subsequent precautionary and preventive measures, as reviewed by the European Environment Agency in Late Lessons from Early Warnings: the Precautionary Principle 1896-2000 (EEA, 2001). In considering the evidence on mobile phones and head cancers the EEA concluded that it would be prudent and timely to issue an "early warning" on the issue, in September, 2007. Five years on, this note briefly updates our opinion on this issue.

II. NEED FOR PRECAUTIONARY ACTIONS ON MOBILE PHONES

The communication leaflet for publication of "Late Lessons from Early Warnings 2: Science, Precaution, Innovation." (EEA, 2012) includes this message:

> *"In the context of scientific uncertainty and ignorance, the decision-makers responsible for incentivising and regulating innovation face a significant challenge in balancing opportunities against risks. The precautionary principle can help to better manage such choices. It requires actions to prevent potentially serious harm before the likelihood or severity of an innovation's impacts become all too clear."*

Volume 2 of 'Late Lessons' includes a chapter on mobile phones and brain tumour risk by Hardell, Carlberg and Gee. Inclusion of a full chapter on the science and public health implications of the mobile phone-brain cancer issue underscores the importance to the European Environmental Agency that mobile phone radiation is a possible health threat. This position is supported by the 2011 classification by the World Health

Organization International Agency for Research on Cancer (IARC) of radiofrequency radiation as a Group 2B Possible Human Carcinogen (Baan et al, 2011).

The evidence in 2012 is stronger than in 2007, and based essentially on two large population studies, the Hardell group in Sweden and the Interphone Study Group which involved 13 countries (WHO Interphone Final Report, 2010; Cardis & Radetski 2010? Hansson Mild et al, 2007; Hardell et al, 2006a, 2006b, 2006c; Hardell et al, 2008; Hardell et al, 2009a, 2009b; Hardell et al, 2010; Hardell et al, 2011a, 2011b; Hardell et al, 2012a in press; Hardell et al, 2012b in press). Are all 12 refs from Hardell needed? Looks like overkill…how about those from 2009?

Some researchers have identified in the last five years "*a consistent pattern of increased risk of glioma and acoustic neuroma associated with use of mobile phones and cordless phones.*" (Hardell et al, 2012b in press), a view that is essentially supported by the leader of the Interphone study. (Cardis & Radetski)

The European Environmental Agency's view on the need for precautionary measures on mobile phones is more warranted in 2012, than it was in 2007, or even early 2011, prior to the IARC decision, when we last reviewed the evidence for a presentation to the Council of Europe (EEA, 2011).

Precautionary actions that can be taken to reduce exposures to RFR would be consistent with actions that have been recommended for other emerging environmental and health issues, for example some uses of the common plastic, BPA, some nanotechnologies, and some food chain additives or contaminants, such as antibiotics, beef hormones, and GMOs. The 25 or so more historical case studies in the 'Late Lessons' volumes such as those on the Minamata Bay disaster, asbestos, leaded petrol, and tobacco illustrate the huge costs of not taking robust early warnings seriously.

Precautionary measures are of particular importance in regard to children, who are generally more biologically sensitive, may be unable to protect themselves; and for whom such exposures may carry greater life-time health risks than they do for adults.

The evidence for a brain tumour risk from mobile phones is still not well established amongst all researchers in the field and there is much scientific controversy about what the current evidence means. The debate is not helped by what might be termed ‘trial by media’ where some scientific advocates leap into the lay press to argue their own case just as, or even before, their research is published. The effects of this behaviour would be minimized if the results of genuine differences of scientific opinion were made transparent when they were published, with clear explanations about the origins of divergent views, such as the scientific paradigms used (“tissue heating” or “information physics” ?); assumptions made; evidence rejected; and values chosen. This does not tend to happen. Divergent scientific views are often smoothed over with the use of what one respected commentator on the reporting of the Interphone results called “oracular “ sentences (Saracci & ?? 2010 ?) which thereby give the media and others the opportunity to report quite opposite conclusions from the same study,as was the case with the Interphone study.

We note that countries including France, Germany, Belgium, Austria, Italy, Russia, India and others have moved toward cautionary warnings and some have revised some target exposure levels for new wireless facilities in line with recommendations issued in 2007. Further actions appear now to be warranted, especially in light of the authoritative 2011 IARC cancer classification.

The IARC, and the EEA , may be wrong to suggest there could be a brain tumour risk from the extensive use of mobile phones, and we dearly hope we are wrong. However, it is worth noting that during over 30 years of classifying cancer risks, covering around 900 agents, IARC very rarely downgrades its judgements: in most cases tentative carcinogens become more certain carcinogens as time since first exposures and further research accumulates. Is it not worth gambling that mobile phones will be one of those very rare cases where IARC has over-classified an agent? We think not. The human cost of getting such a gamble wrong would be too great, especially in light of the relatively low cost of reducing exposures significantly.

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SECTION 24

Key Scientific Evidence and Public Health Policy Recommendations

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I. KEY SCIENTIFIC EVIDENCE

Exposure to electromagnetic fields (EMF) has been linked to a variety of adverse health outcomes. The health endpoints that have been reported to be associated with ELF and/or RF include childhood leukemia, adult brain tumors, childhood brain tumors, genotoxic effects (DNA damage and micronucleation), neurological effects and neurodegenerative disease, immune system disregulation, allergic and inflammatory responses, breast cancer in men and women, miscarriage and some cardiovascular effects.

Effects are not specifically segregated for ELF or RF, since many overlapping exposures occur in daily life; and because this is an artificial division based on frequencies as defined in physics that has little bearing on the biological effects. Both ELF and RF, for example have been shown to cause cells to generate stress proteins, a universal sign of distress in plant, animal and human cells.

The number of people exposed to elevated levels of EMF has been estimated in various studies, and there is general agreement among them. In the United States, few people have chronic or prolonged exposures over 4 mG (0.4 µT) (Kheifets et al, 2005b). Section 20 has information on average residential and occupational ELF levels. The highest exposure category in most all studies is ≥ 4 mG (≥ 0.4 µT). Many people have daily exposures to ELF in various ways, some of them up to several hundred milligauss for short periods of time, but relatively few people with the exception of some occupational workers habitually experience ELF exposures greater than 1-2 mG (0.2 – 0.3 µT - App. 20-A).

The exposure of children to EMF has not been studied extensively; in fact, the FCC standards for exposure to radiofrequency radiation are based on the height, weight and stature of a 6-foot tall man, not scaled to children or adults of smaller stature. They do not take into account the unique susceptibility of growing children to exposures (SCENIHR, 2007; Jarosinska and Gee, 2007), nor are there studies of particular relevance to children.

Differences in exposure patterns between infants, children and adults; 2) special susceptibilities of infants and children to the effects of EMF; and 3) interactions between chemical contaminants and EMF are lacking; as are studies on chronic exposure for both children and adults. There is reason to believe that children may be more susceptible to the effects of EMF exposure since they are growing, their rate of cellular activity and division is more rapid, and they may be more at risk for DNA damage and subsequent cancers. Growth and development of the central nervous system is still occurring well into the teenage years so that neurological changes may be of great importance to normal development, cognition, learning, and behavior. Prenatal exposure to EMF have been identified as possible risk factor for childhood leukemia. Children are largely unable to remove themselves from exposures to harmful substances in their environments. Their exposure is involuntary.

Like second-hand smoke, EMF is a complex mixture, where different frequencies, intensities, durations of exposure(s), modulation, waveform and other factors is known to produce variable effects. Many years of scientific study has produced substantial evidence that EMF may be considered to be both carcinogenic and neurotoxic. The weight of evidence is discussed in this report, including epidemiological evidence and studies on laboratory animals.

Relative risk estimates associated with some of these endpoints are small and the disease is fairly rare (for childhood leukemia, for example), For other diseases, the risk estimates are small but the diseases are common and EMF exposures at levels associated with increased risks are widespread and chronic so the overall public health impacts may be very large.

A. Weight of Evidence Assessment and Criteria for Causality

A weight-of-evidence approach has been used to describe the body of evidence between health endpoints and exposure to electromagnetic fields (ELF and RF).

The number and quality of epidemiological studies, as well as other sources of data on biological plausibility are considered in making scientific and public health policy judgments. Methodological issues that were considered in the review of the epidemiological literature include 1) quality of exposure assessment. 2) sample size of the study, which detects the power to detect an effect, 3) extent to which the analysis or design takes into account potential confounders or other risk factors, 4) selection bias, 5) the potential for bias in determining exposure. Assessment of the epidemiological literature is consistent with guidelines from Hill (1971), Rothman and Greenland (1998) and the Surgeon General's Reports on Smoking (US DHHS, 2004), and California Air Resources Board (2005). Factors that were considered in reaching conclusions about the weight of evidence overall included strength of the association, consistency of association, temporality, biological plausibility, dose-response and issues with non-linear dose-response, specificity and experimental evidence.

There is a relatively large amount of human epidemiological information with real world exposures, including data from occupational studies. There is less animal data in most cases, except for the genotoxicity studies. Human epidemiological evidence has be given the greatest weight in making judgments about weight-of-evidence, where the results across high quality studies give relatively consistent positive results. Meta-analyses of childhood leukemia, adult leukemia, adult brain tumors, childhood brain tumors, male and female breast cancer and Alzheimer's disease were relied upon in assessing the overall strength of epidemiological study results. Sections 5 – 15 provide analysis of the relevant scientific studies that are key evidence in making public health policy recommendations with respect to exposure to electromagnetic fields (both ELF and RF).

B. Summary of Evidence

1. Childhood Leukemia

Several meta-analyses have been conducted to assess risks of childhood leukemia from exposure to ELF. The results of these studies that combine or pool results of many individual studies (including studies that report both effects and no effects) consistently report increased risks.

Meta-Analysis: Studies of Childhood Leukemia and EMF

Greenland et al., (2000) reported a significantly elevated risk of 1.68 [95% CI 1.23-2.31] based on pooled results from 12 studies using a time-weighted average of exposure greater than 3 mG (0.3 µT). This is a 68% increased risk of childhood leukemia.

Ahlbom et al., (2000) reported a doubling of risk based on a meta-analysis of nine (9) studies. The results reported an elevated risk of 2.0 [95% CI 1.27-3.13] for EMF exposures equal to or greater than 4 mG (0.4 µT) as compared to less than 1 mG (0.1 µT)

Other Relevant Evidence

In 2002, the International Agency for Cancer Research (IARC) designated EMF as a "possible human carcinogen" or Group 2B Carcinogen based on consistent epidemiological evidence. The exposure levels at which increased risks of childhood leukemia are reported in individual studies range from above 1.4 mG or 0.14 µT (Green et al., 1999)for younger children to age six (6) to 4 mG (0.4 µT). Many individual studies with cutpoints of 2 mG or 3 mG (0.2-0.3 µT)) report increased risks. Plausible biological mechanisms exist that may reasonably account for a causal relationship between EMF exposure and childhood leukemia.

Recurrence of Childhood Leukemia and Poorer Survival Rates with Continued EMF Exposure

Foliart reported more than a four-fold (450% increased risk) of adverse outcome (poorer survival rate) for children with acute lymphoblastic leukemia (ALL) who were recovering in EMF environments of 3 mG (0.3 µT) and above (OR 4.5, CI 1.5-13.8). Svendsen reported a poorer survival rate of children with acute lymphoblastic leukemia (ALL) in children exposed to 2 mG (0.2 µT) and above. These children were three times more likely (300% increased risk) to die than children recovering in fields of less than 1 mG (OR 3.0, CI 0.9.8). Children recovering in EMF environments between 1- 2 mG (0.1-0.2 µT) also had poorer survival rates, where the increased risk was 280% (OR 2.8, CI 1.2-6.2).

Higher Lifetime Cancer Risks with Childhood EMF Exposure

Lowenthal (2007) reported that children raised for the first five years in home environments exposed to EMF within 300 meters of a high voltage power line have a five-fold (a 500 percent increased risk of developing some kinds of cancers sometime in later life. For children from newborn to 15 years of age; it is a three-fold risk of developing cancer later in life (Lowenthal et al., 2007). There is suggestive evidence for a link between adult leukemia and EMF exposure.

Attributable Risk

Wartenberg estimates that 8% to 11% of childhood leukemia cases may be related to ELF exposure. This translates into an additional 175 to 240 cases of childhood leukemia based on 2200 US cases per year. The worldwide total of annual childhood leukemias is estimated to be 49,000, giving an estimate of nearly 4000 to 5400 cases per year. Other researchers have estimated higher numbers that could reach to 80% of all cases (Milham, 2001).

2. Childhood Brain Tumors

Childhood Brain Tumors

There is suggestive evidence that other childhood cancers may be related to EMF exposure. The meta-analysis by Wartenberg et al., (1998) reported increased risks for childhood brain tumors. Risks are quite similar whether based on calculated EMF fields (OR = 1.4, 95% CI = 0.8 – 2.3] or based on measured EMF fields (OR = 1.4, 95% CI = 0.8 – 2.4).

3. Adult Brain Tumors

Brain Tumors in Electrical Workers and in Electrical Occupations (Meta-analysis)

A significant excess risk for adult brain tumors in electrical workers and those adults with occupational EMF exposure was reported (Kheifets et al., 1995). This is about the same size risk for lung cancer and second hand smoke (US DHHS, 2006). A total of 29 studies with populations from 12 countries were included in this meta-analysis. The relative risk was reported as 1.16 (CI = 1.08 – 1.24) or a 16% increased risk for all brain tumors. For gliomas, the risk estimate was reported to be 1.39 (1.07 – 1.82) or a 39% increased risk for those in electrical occupations. A second meta-analysis published by Kheifets et al., ((2001) added results of 9 new studies published after 1995. It reported a new pooled estimate (OR = 1.16, 1.08 – 1.01) that showed little change in the risk estimate overall from 1995.

4. Brain Tumors and Acoustic Neuromas in Cell Phone and Cordless Phone Users (Meta-Analysis)

Glioma and Acoustic Neuroma

Hardell et al., (2007) reported in a meta-analysis statistically significant increased risk for glioma with exposure of 10 years or greater in persons using cell phones. Risks were estimated to be 1.2 (0.8 – 1.9) for all use; but when ipsilateral use was assessed (mainly on same side of head) it increased the risk of glioma to 2.0 (1.2 – 3.4) for 10 years and greater use.

For acoustic neuromas, Hardell et al., (2007) reported the increased risk with 10 years or more of exposure to a cell phone at 1.3 (0.6 – 2.8) but this risk increased to 2.4 (1.1 – 5.3) with ipsilateral use (mainly on the same side of the head). There is a consistent pattern of increased risk for brain tumors (glioma) and acoustic neuromas at 10 years and greater exposure to cell phones.

The meta-analysis by Lakhola et al., (2006) reported that brain tumor risk was 1.3 (0.99 – 1.9) for ipsilateral use of a cell phone, but no data was given for exposures at 10 years or greater (all exposures were of shorter duration).

The meta-analysis by Kan et al., (2007) reported "no overall risk" but found elevated risk of brain tumors (RR = 1.25, CI 1.01 – 1.54) $\geq$ 10 years, reinforcing the findings of other pooled estimates of risk. No estimates of increased risk with ipsilateral use were provided, which would have likely increased reported risks.

5. Neurodegenerative Diseases

Alzheimer's Disease and ALS

Evidence for a relationship between exposure and the neurodegenerative diseases, Alzheimer's and amyotrophic lateral sclerosis (ALS), is strong and relatively consistent. While not every publication shows a statistically significant relationship between exposure and disease, ORs of 2.3 (95% CI = 1.0-5.1 in Qio et al., 2004), of 2.3 (95% CI = 1.6-3.3 in Feychting et al., 2003) and of 4.0 (95% CI = 1.4-11.7 in Hakansson et al., 2003) for Alzheimer's Disease.

Hakansson et al., report more than a doubling of risk for ALS 2.2 (95% CI = 1.0-4.7).

Savitz et al., (1998) reports more than a tripling of risk for ALS (3.1, CI = 1.0 – 9.8).

6. Breast Cancer (Men and Women)

A meta-analysis by Erren (2001) on EMF and breast cancer reported pooled relative risks based on studies of both men and women. A total of 38 publications were reviewed; there were 23 studies on men; 25 studies on women; and 10 studies on both men and women. The pooled relative risk for women exposed to EMF was 1.12 (CI 1.09 – 1.15) or a 12% increased risk, Erren observed that variations between the contributing results are not easily attributable to chance (P = 0.0365). For men and breast cancer, he reported a fairly homogeneous increased risk (a pooled relative risk of 1.37 [CI 1.11 – 1.71]*)*.

This analysis is well conducted. The results were stratified according to measured or assumed intensity of exposure to EMF; and the estimate of risk for the most heavily exposed group was extracted. Independent estimates of RRs were grouped according to gender, type of study (case-control and cohort), country where the study was conducted and method used to assess exposure. Pooled estimates of RRs and their 95% confidence intervals (CI) referring to various combinations of these factors were calculated according to appropriate statistical methods (Greenland, 1987). Misclassification possibilities were thoroughly assessed, and whether the results were sole endpoints or there were multiple endpoints in each study did not affect the RRs.

Erren qualifies his findings by discussing that latencies for cancers can be 20 to 30 years, Further, he notes that studies of total EMF exposures from both home, travel and workplace are rarely available, and these EMF sources are ubiquitous. Both could result in underestimation of risks. Another way in which risks might be masked is by variations in age of study participants. Forssen and colleagues (2000) reported no increased RRs for breast cancer in women of all ages when they combined residential and occupational EMF exposures (RR = 0.9, CI 0.3 – 2.7). However, when risks for the women younger than 50 years of age were separated out and calculated, the RR increased to 7.3 (CI 0.7 – 78.3) although with wide confidence intervals based on only four cases. Erren notes

"When possibly relevant exposures to EMF in the whole environment are assessed only partially, errors in the categorization of exposure status are likely to occur. If such misclassification is random and thus similar in subrgroups being compared (nondifferential), then the error will tend to introduce bias towards the null. Substantial random misclassification of exposures would then tend to generate spurious reports of 'little or no effect'. Note for example that estimates of smoking-associated lung cancer risks in the early 1950's could have been seriously distorted if exposure assessment had not considered smoking either at work or at home."

"Collectively, the data are consistent with the idea that exposures to EMF, as defined, are associated with some increase in breast cancer risks, albeit the excess risk is small." Erren (2001)

7. Combined Effects of Toxic Agents and ELF

ELF and Toxic Chemical Exposures

There is also the issue of what weight to give the evidence for synergistic effects of toxic chemical exposure and EMF exposure. Juuilainen et al., (2006) reported that the combined effects of toxic agents and ELF magnetic fields together enhances damage as compared to the toxic exposure alone. In a meta-analysis of 65 studies; overall results showed 91% of the *in vivo* studies and 68% of the *in vitro* studies had worse outcomes (were positive for changes indicating synergistic damage) with ELF exposure in combination with toxic agents. The percentage of the 65 studies with positive effects was highest when the EMF exposure preceded the other exposure. The radical pair mechanism (oxidative damage due to free radicals) is cited as a good candidate to explain these results. Reconsideration of exposure limits for ELF is warranted based on this evidence.

II. FALLACIES AND ANSWERS IN THE DEBATE OVER EMF EVIDENCE

There are several arguments (false, in our view) that have been presented by those who minimize the strength of the relationship between exposure to both 50-60Hz ELF and RF EMFs. These are as follows:

A. “Only a small number of children are affected.”

This argument is not correct because we do not know precisely how many children are affected. In 1988 Carpenter and Ahlbom attempted to answer this question based on the results of the New York State Powerlines Project and the results of the study of Savitz et al. (1988), and concluded that if the magnetic fields homes in the US were similar to those in Denver, Colorado fully 10 to 15% of US childhood leukemia (about 1,000 cases) could be associated with residential magnetic field exposure. They then concluded that exposure to magnetic fields from non-residential sources (particularly appliances) must be at least equal in magnitude, and that if so these two sources of exposure would account for 20-35% of childhood leukemia.

There have been several meta-analyses of the childhood leukemia data (Wartenberg, 1998; Greenland et al., 2000; Ahlbom et al., 2000). All have concluded that there is a significant association between residential exposure to magnetic fields and elevated risk of leukemia in children. Greenland et al. (2000) performed a meta-analysis of 15 studies of magnetic field or wire code investigations of childhood leukemia, and calculated the attributable fraction of cases of childhood leukemia from residential magnetic field exposure in the US was 3%. Ahlbom et al. (2000) conducted a different meta-analysis that concluded there was a significant 2-fold elevation of risk at exposure levels of 4 mG (0.4 μT) or greater. Kheifets et al. (2006) attempted to calculate the attributable fraction of worldwide childhood leukemia due to EMFs, based on the meta-analyses of Ahlbom et al. (2000) and Greenland et al., (2000). They concluded that the attributable fraction of leukemia was between <1% to 4%. The recent WHO Environmental Health Criteria ELF Monograph #238 (2007) states “(A)ssuming that the association is causal, the number of cases of childhood leukaemia worldwide that might be attributable to exposure can be estimated to range from 100 to 2,400 cases per year. However this represents 0.2 to 4.9% of the total annual incidence of leukaemia cases, estimated to be 49,000 worldwide in 2000. Thus, in a global context, the impact on public health, if any, would be limited and uncertain.”

These reports are important, in that they show consistency in there being a clearly elevated risk of leukemia in children with EMF exposure from power line fields in homes. These meta-analyses lead to the conclusion, reflected in the WHO report, that there is an association between childhood cancer and exposure to elevated magnetic fields in homes. We strongly disagree, however, with the overall conclusion that these calculations indicate that the fraction of childhood leukemia attributable to EMFs is so small as to not have serious public health implications.

There are several reasons why the WHO ELF Environmental Health Criteria Monograph conclusion is not justified. These studies all considered either only measured magnetic fields in homes or wire codes from power lines, ignoring exposure from appliances, wireless devices and all exposures outside of the home. Thus these metrics do not come close to accounting for any individual’s cumulative exposure to EMFs. If residential magnetic fields cause cancer, then those from other sources will add to the risk. The failure to measure total EMF exposure would tend to obscure the relationship and lead to gross underestimation of the true relationship between exposure and disease. While the evidence for a relationship between exposure and childhood leukemia may be considered to be definitive at exposure levels of 3 or 4 mG (0.3 or 0.4 µT) or higher; there is evidence from some (but not all) of the other studies for an elevated risk at levels not greater than 2 mG (0.2 µT) (Savitz et al., 1988; Green, 1999). There is absolutely no evidence that exposures at lower levels are "safe", since persons with these exposures are usually the "control" group. Therefore this WHO statement fails to acknowledge the true magnitude of the problem, even when considering only childhood leukemia. The global attributable risk of childhood leukemia as a result of exposure to EMFs must be significantly greater than that calculated from consideration of only residential 50/60 Hz magnetic fields in studies where there is no unexposed control.

As detailed in other chapters in this report (Chapter 10), there is some evidence for a relationship between EMF exposure and brain cancers in children. We have almost no understanding of the mechanisms behind the development of brain cancers, and any cancer in a child is a tragedy. While evidence for a relationship between EMF exposure and childhood brain cancer is not as strong as for leukemia, it is of concern and deserves more study. Of even greater concern, given the clear evidence for elevated risk of childhood leukemia upon exposure to 50/60 Hz EMFs, is the relative lack of a comparable body of information on the effects of radiofrequency EMFs on the health of children. A recent study of South Korean children (1,928 with leukemia, 956 with brain cancer and 3,082 controls) living near to AM radio transmitters reports an OR of 2.15 (95% CI = 1.19-2.11) for risk of leukemia in children living within 2 km of the nearest AM transmitter as compared to those living more than 20 km from it (Ha et al., 2007). No relation was found for brain cancer. This study is consistent with the hypothesis that radiofrequency EMFs have similar effects to 50/60 Hz EMFs, but more study is needed. Since radiofrequency EMFs have higher energy than do power line frequencies, one might expect that they would be even more likely to cause disease. The enormous and very recent increase in use of cell phones by children is particularly worrisome. However there is little information at present on the long-term consequences of cell phone use, especially by children.

B. "There is insufficient evidence that adult diseases are secondary to EMF exposure."

It is correct that the level of evidence definitively proving an association between exposure to EMFs and various adult diseases is less strong that the relationship with childhood leukemia. However there are multiple studies which show statistically significant relationships between occupational exposure and leukemia in adults (see Chapter 11), in spite of major limitations in the exposure assessment. A very recent study by Lowenthal et al. (2007) investigated leukemia in adults in relation to residence near to high-voltage power lines. While they found elevated risk in all adults living near to the high voltage power lines, they found an OR of 3.23 (95% CI = 1.26-8.29) for individuals who spent the first 15 years of life within 300 m of the power line. This study provides support for two important conclusions: adult leukemia is also associated with

EMF exposure, and exposure during childhood increases risk of adult disease. Thus protecting children from exposure should be a priority.

The evidence for a relationship between exposure and breast cancer is relatively strong in men (Erren, 2001), and some (by no means all) studies show female breast cancer also to be elevated with increased exposure (see Chapter 12). Brain tumors and acoustic neuromas are more common in exposed persons (see Chapter 10). There is less published evidence on other cancers, but Charles et al. (2003) report that workers in the highest 10% category for EMF exposure were twice as likely to die of prostate cancer as those exposed at lower levels (OR 2.02, 95% CI = 1.34-3.04). Villeneuve et al. (2000) report statistically significant elevations of non-Hodgkin's lymphoma in electric utility workers in relation to EMF exposure, while Tynes et al. (2003) report elevated rates of malignant melanoma in persons living near to high voltage power lines. While these observations need replication, they suggest a relationship between exposure and cancer in adults beyond leukemia.

Evidence for a relationship between exposure and the neurodegenerative diseases, Alzheimer's and amyotrophic lateral sclerosis (ALS), is strong and relatively consistent (see Chapter 12). While not every publication shows a statistically significant relationship between exposure and disease, ORs of 2.3 (95% CI = 1.0-5.1 in Qio et al., 2004), of 2.3 (95% CI = 1.6-3.3 in Feychting et al., 2003) and of 4.0 (95% CI = 1.4-11.7 in Hakansson et al., 2003) for Alzheimer's Disease, and of 3.1 (95% CI = 1.0-9.8 in Savitz et al., 1998) and 2.2 (95% CI = 1.0-4.7 in Hakansson et al., 2003) for ALS cannot be simply ignored.

In total the scientific evidence for adult disease associated with EMF exposure, given all of the difficulties in exposure assessment, is sufficiently strong that preventive steps are appropriate, even if not all reports have shown exactly the same positive relationship. While there are many possible sources of false positive results in epidemiological studies, there are even more possible reasons for false negative results, depending on sample size, exposure assessment and a variety of other confounders. It is inappropriate to discount the positive studies just because not every investigation shows a positive result. While further research is needed, with better exposure assessment and control of confounders; the evidence for a relationship between EMF exposure and adult cancers and neurodegenerative diseases is sufficiently strong at present to merit preventive actions to reduce EMF exposure.

C. "The risk is low."

This argument is incorrect because at present it is not possible to determine the magnitude of the risk. Clearly as far as EMFs are concerned there is no unexposed population. Therefore one can only compare groups with different levels of exposure. We can perhaps say with confidence that the elevated risk of leukemia from residential exposure of children to magnetic fields is "low" (meaning ORs in the range of 2-4), but this does not consider the child's exposure to appliances, exposure in automobiles and at daycare or school, exposures in playgrounds and at all of the other places that a child spends time. Even if the risk to one individual is low, the societal impact when everyone is exposed may be very significant.

In addition the exposure assessment is grossly inadequate, even in the best of studies. Most reports deal only with either characterization of the fields within residences or with job titles in occupational settings. Some studies attempt to quantitate other sources of exposure, such as frequency of cell phone usage or use of other appliances, but these studies almost always do not consider residential exposure from power lines. In no investigation has it been possible to follow the exposures of a large number of people over a number of years with accurate monitoring of total exposure to EMFs. This would of course be almost impossible to do for the very good reason that as a person moves through his or her environment the exposures vary from place to place and from moment to moment. However to truly and objectively determine the risk of exposure to EMFs it is essential to consider residential, occupational (or school) and recreational exposures to the full range of the electromagnetic spectrum, including appliances and wireless devices. This has not been accomplished in any study, and without such information it is not possible to determine the overall magnitude of the risk. It is possible, indeed likely, that upon consideration of both childhood and adult diseases that the risk is not low.

D. “There is no animal evidence”.

It is correct that there is no adequate animal model system that reproducibly demonstrates the development of cancer in response to exposure to EMFs at the various frequencies of concern. McCann et al. (1997) reviewed the animal studies, and while they found most to be negative there were several that showed suggestive positive results. They also clearly identified issues that need to be improved in further animal carcinogenesis investigations. However Kheifets et al. (2005a) in a policy review noted that “even consistent negative toxicological data cannot completely overcome consistent epidemiological studies. First, a good animal model for childhood leukemia has been lacking. Second, particularly for ELF, the complex exposures that humans encounter on a daily basis and a lack of understanding of the biologically relevant exposure calls into question the relevance of exposures applied in toxicology. Another limitation of toxicologic studies is that animals cannot be exposed to fields that are orders of magnitude more powerful than those encountered by humans, decreasing their power to detect small risks.” Further, they conclude that “(A)lthough the body of evidence is always considered as a whole, based on the weight of evidence approach and incorporating different lines of scientific enquiry, epidemiologic evidence, as most relevant, is given the greatest weight.”

One positive animal study is that by Rapacholi et al. (1997), who demonstrated that lymphoma-prone transgenic mice developed significantly more lymphoma after exposure to 900 MHz fields (lymphoma being the animal equivalent of human leukemia) than did unexposed animals. More striking is the report from Denver, Colorado using the wire-code characterization originally developed by Wertheimer and Leeper (1979) showing that pet dogs living in homes characterized as having high or very high wire codes, as compared to those with low or very low wire codes or buried power lines, showed a OR of 1.8 (95% CI = 0.9-3.4) for development of lymphoma after adjustment for potential confounders, whereas dogs that lived in homes with very high wire codes had an OR of 6.8 (95% CI = 1.6-28.5) (Reif et al., 1995). This study is impressive because the exposure of the dogs reflects the environment in which exposure has been associated with elevated risk of human cancer in two independent investigations (Wertheimer and Leeper, 1979: Savitz et al., 1988).

It is curious that in many legal situations the courts are reluctant to accept only evidence that substance X causes cancer in animals without corresponding evidence in humans. In the case of EMFs we have strong evidence that EMFs cause cancer in human, but much less evidence from animal models. The US Supreme Court, in the case of Daubert vs. Merrell Dow Pharmaceuticals, effectively ruled that animal studies were not relevant to human health, and that the only admissible evidence must be from human epidemiological studies! While this is certainly not a justifiable conclusion, the situation with regards to EMF health effects is that we have strong evidence for human cancer from epidemiological studies, but do not have good evidence for cancer in experimental animals. But it is humans that we should be concerned about, not the laboratory rats.

E. "We do not know a mechanism."

We do not know the mechanism of cancer in general, although we know a lot about cancer. It came as a major surprise to most scientists when Lichtenstein et al., (2000) reported that genetic factors play a minor role in causing most types of cancer, since it was commonly assumed that genetics was the major cause. However Lichtenstein et al. concluded from their study of identical twins that environmental factors were the initiating event in the great majority of cancers. This does not, of course, mean that genetic susceptibility to environmental contaminants is unimportant, but only that genetic factors alone do not result in cancer. We know mechanisms of action for some carcinogenic substances, but for most cancers we know neither the environmental trigger nor the mechanism of action. So there is no reason to negate the evidence that EMFs cause cancer just because we do not know a single mechanism to explain it's mode of action.

We do not know the mechanism or cause for development of Alzheimer's Disease or ALS. We do know that both are more common in individuals in certain occupations, and that exposure to certain metals appears to be associated with increased risk (Kamel et al., 2002; Shcherbatykh and Carpenter, 2007). In the case of Alzheimer's Disease there are abnormalities of amyloid β and tau protein (Goedert and Spillantini, 2006), but very limited understanding of why or how they form. Neither the association with metals nor the presence of abnormal proteins constitutes a mechanism for cause of disease. So rather than discounting the relationship between EMF exposure and neurodegenerative diseases we should be using this information as a tool to better understand the etiology of these diseases.

There is clear evidence from animal and cell culture studies that ELF and RFR have biological effects. Furthermore, these effects occur at intensities commonly experienced by humans. We know a number of ways in which EMFs alter cell physiology and function, as detailed in various chapters in this report. EMFs affect gene transcription (Chapter 5 and 6), cause the synthesis of stress proteins (Chapter 7) and cause breakage of DNA, probably through generation of reactive oxygen species (Chapter 6 and 9 - Lai and Singh, 2004). Any one of these actions might be responsible for the carcinogenic and neurodegenerative actions of EMFs. However, as with many environmental agents, it would be a mistake to assume that there is only one target or mechanism of action. It is unlikely, for example, that the effects on the nervous system and behavior are secondary to exactly the same cellular targets and actions that lead to cancer. It is likely that there are multiple mechanisms of action leading to disease. But the lack of complete understanding of basic mechanisms does not alter the importance of the relationships.

F. Vested Interests: How They Shape the Public Health Debate

There is no question but that global implementation of the safety standards proposed in this report has the potential to not only be very expensive but also could be disruptive of life and economy as we know it if implemented abruptly and without careful planning. Action must be a balance of risk to cost to benefit. However, "deny and deploy" strategies by industry should not be rewarded in future risk assessment calculations. For example, if significant economic investments in the roll-out of risky technologies persist beyond the time that there is reasonable suspicion of risk available to all who look, then such costs should not be borne by ratepayers (in the case of new powerlines) or by compensating industry for bad corporate choices. Such investments in the deployment of new sources of exposure for ELF and RF should not count toward the balance sheet when regulatory agencies perform risk assessments. Mistakes may be made, but industry should make mid-course corrections to inform and protect the public, rather than deny effects pending "proof". Whether the costs of remedial action are worth the societal benefits is a formula that should reward precautionary behavior. Prudent corporate policies should be expected to address and avoid future risks and liabilities. Otherwise, there is no market incentive to produce safe (and safer) products.

The deployment of new technologies is running ahead of any reasonable estimation of possible health impacts and estimates of probabilities, let alone a solid assessment of risk. However what has been missing with regard to EMF has been an acknowledgement of the risk that is demonstrated by the scientific studies. As discussed in earlier sections, in this case there is clear evidence of risk, although the magnitude of the risk is uncertain, and the magnitude of doing nothing on the health effects cost to society is similarly uncertain. This situation is very similar to our history of dealing with the hazards of smoking decades ago, where the power of the industry to influence governments and even conflicts of interest within the public health community delayed action for more than a generation, with consequent loss of life and enormous extra health care costs to society.

> Just because a problem is difficult to solve is not a reason to deny that a problem exists. In fact solutions to difficult issues usually can't be expected until the issues are known and creative thinking is brought to bear to find a solution.
>
> The most contentious issue regarding public and occupational exposures to ELF and RF involves the resolute adherence to existing ICNIRP and IEEE standards by many countries, in the face of growing scientific evidence of health risks at far lower levels. Furthermore there is widespread belief that governments are ignoring this evidence. There are two obvious factors that work against governments taking action to set exposure guidelines based on current scientific evidence of risk. These are: 1) contemporary societies are very dependent upon electricity usage and RF communications, and anything that restricts current and future usage potentially has serious economic consequences and 2) the electric power and communications industries have enormous political clout and even provide support for a significant fraction of what research is done on EMF. This results in legislation that protects the status quo and scientific publications whose conclusions are not always based on only the observations of the research. It hinders wise public health policy actions and implementation of prevention strategies because of the huge financial investments already made in these technologies.

In 1989, in an editorial for Science Magazine, Philip H. Abelson called for more research into low-frequency electromagnetic fields. At that time, he confirmed that a US Office of Technology Assessment (OTA) study had determined that *"(o)verall, the evidence is too weak to allow firm conclusions either way*" but a policy of prudent avoidance strategy was suggested, Abelson defined this as "*to systematically look for strategies which can keep people out of 60 Hz fields*". Both policy actions were developed in the midst of scientific uncertainty, but rising concern for possible health impacts to the public. At that time, with high level of unknowns, the appropriate level of policy action was prudent avoidance or precautionary action. Nearly two decades later, the level of action warranted is higher – based on many new scientific publications confirming risks may exist – and justifying prevention or preventative action.

III. EMF EXPOSURE AND PRUDENT PUBLIC HEALTH PLANNING

- *The scientific evidence is sufficient to warrant regulatory action for ELF; and it is substantial enough to warrant preventative actions for RF.*

- *The standard of evidence for judging the emerging scientific evidence necessary to take action should be proportionate to the impacts on health and well-being*

- *The exposures are widespread.*

- *Widely accepted standards for judging the science are used in this assessment.*

Public exposure to electromagnetic radiation (power-line frequencies, radiofrequency and microwave) is growing exponentially worldwide. There is a rapid increase in electrification in developing countries, even in rural areas. Most members of society now have and use cordless phones, cellular phones, and pagers. In addition, most populations are also exposed to antennas in communities designed to transmit wireless RF signals. Some developing countries have even given up running land lines because of expense and the easy access to cell phones. Long-term and cumulative exposure to such massively increased RF has no precedent in human history. Furthermore, the most pronounced change is for children, who now routinely spend hours each day on the cell phone. Everyone is exposed to a greater or lesser extent. No one can avoid exposure, since even if they live on a mountain-top without electricity there will likely be exposure to communication-frequency RF exposure. Vulnerable populations (pregnant women, very young children, elderly persons, the poor) are exposed to the same degree as the general population. Therefore it is imperative to consider ways in which to evaluate risk and reduce exposure. Good public health policy requires preventative action proportionate to the potential risk of harm and the public health consequence of taking no action.

IV. RECOMMENDED ACTIONS

A. Defining new exposure standards for ELF

This chapter concludes that new ELF limits are warranted based on a public health analysis of the overall existing scientific evidence. The public health view is that new ELF limits are needed now. They should reflect environmental levels of ELF that have been demonstrated to increase risk for childhood leukemia, and possibly other cancers and neurological diseases. ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor. It is no longer acceptable to build new power lines and electrical facilities that place people in ELF environments that have been determined to be risky. These levels are in the 2 to 4 milligauss* (mG) range (0.2 – 0.4 µT), not in the 10s of mG or 100s of mG. The existing ICNIRP limit is 1000 mG (100 µT) and 904 mG (90.4 µT) in the US for ELF is outdated and based on faulty assumptions. These limits are can no longer be said to be protective of public health and they should be replaced. A safety buffer or safety factor should also be applied to a new, biologically-based ELF limit, and the conventional approach is to add a safety factor lower than the risk level.

While new ELF limits are being developed and implemented, a reasonable approach would be a 1 mG (0.1 µT) planning limit for habitable space adjacent to all new or upgraded power lines and a 2 mG (0.2 µT) limit for all other new construction. It is also recommended for that a 1 mG (0.1 µT) limit be established for existing habitable space for children and/or women who are pregnant (because of the possible link between childhood leukemia and *in utero* exposure to ELF). This recommendation is based on the assumption that a higher burden of protection is required for children who cannot protect themselves, and who are at risk for childhood leukemia at rates that are traditionally high enough to trigger regulatory action. This situation in particular warrants extending the 1 mG (0.1 µT) limit to existing occupied space. "Establish" in this case probably means formal public advisories from relevant health agencies. While it is not realistic to reconstruct all existing electrical distribution systems, in the short term; steps to reduce exposure from these existing systems need to be initiated, especially in places where children spend time, and should be encouraged. These limits should reflect the exposures that are commonly associated with increased risk of child hood leukemia (in the 2 to 5 mG (0.2 to 0.5 µT) range for all children, and over 1.4 mG (0.14 µT) for children age 6 and younger). Nearly all of the occupational studies for adult cancers and neurological diseases report their highest exposure category is

4 mG (0.4 µT) and above, so that new ELF limits should target the exposure ranges of interest, and not necessarily higher ranges.

Avoiding chronic ELF exposure in schools, homes and the workplace above levels associated with increased risk of disease will also avoid most of the possible bioactive parameters of ELF discussed in the relevant literature.

It is not prudent public health policy to wait any longer to adopt new public safety limits for ELF. These limits should reflect the exposures that are commonly associated with increased risk of childhood leukemia (in the 2 to 5 mG (0.2-0.5 µT) range for all children, and over 1.4 mG (0.14 µT) for children age 6 and younger). Avoiding chronic ELF exposure in schools, homes and the workplace above levels associated with increased risk of disease will also avoid most of the possible bioactive parameters of ELF discussed in the relevant literature.

B. Defining preventative actions for reduction in RF exposures

Given the scientific evidence at hand, the rapid deployment of new wireless technologies that chronically expose people to pulsed RF at levels reported to cause bioeffects, whicih in turn, could reasonably be presumed to lead to serious health impacts, is a public health concern. A public health action level that implements preventative action now is warranted, based on the collective evidence. There is suggestive to strongly suggestive evidence that RF exposures may cause changes in cell membrane function, cell communication, metabolism, activation of proto-oncogenes and can trigger the production of stress proteins at exposure levels below current regulatory limits. Resulting effects can include DNA breaks and chromosome aberrations, cell death including death of brain neurons, increased free radical production, activation of the endogenous opioid system, cell stress and premature aging, changes in brain function including memory loss, retarded learning, performance impairment in children, headaches and fatigue, sleep disorders, neurodegenerative conditions, reduction in melatonin secretion and cancers (Chapters 5, 6, 7, 8, 9, 10, and 12).

As early as 2000, some experts in bioelectromagnetics promoted a 0.1 µW/cm2 limit (which is 0.614 Volts per meter) for ambient outdoor exposure to pulsed RF, so generally in cities, the public would have adequate protection against involuntary exposure to pulsed radiofrequency (e.g., from cell towers, and other wireless technologies). The Salzburg Resolution of 2000 set a target of 0.1 µW/cm2 (or 0.614 V/m) for public exposure to pulsed radiofrequency. Since then, there are many credible anecdotal reports of unwellness and illness in the vicinity of wireless transmitters (wireless voice and data communication antennas) at lower levels. Effects include sleep disruption, impairment of memory and concentration, fatigue, headache, skin disorders, visual symptoms (floaters), nausea, loss of appetite, tinnitus, and cardiac problems (racing heartbeat), There are some credible articles from researchers reporting that cell tower -level RF exposures (estimated to be between 0.01 and 0.5 µW/cm2) produce ill-effects in populations living up to several hundred meters from wireless antenna sites,

This information now argues for thresholds or guidelines that are substantially below current FCC and ICNIPR standards for whole body exposure. Uncertainty about how low such standards might have to go to be prudent from a public health standpoint should not prevent reasonable efforts to respond to the information at hand. No lower limit for bioeffects and adverse health effects from RF has been established, so the possible health risks of wireless WLAN and WI-FI systems, for example, will require further research and no assertion of safety at any level of wireless exposure (chronic exposure) can be made at this time. The lower limit for reported human health effects has dropped 100-fold below the safety standard (for mobile phones and PDAs); 1000- to 10,000-fold for other wireless (cell towers at distance; WI-FI and WLAN devices). The entire basis for safety standards is called into question, and it is not unreasonable to question the safety of RF at any level.

A cautionary target level for pulsed RF exposures for ambient wireless that could be applied to RF sources from cell tower antennas, WI-FI, WI-MAX and other similar sources is proposed. The recommended cautionary target level is 0.1 microwatts per centimeter squared (µW/cm2)** (or 0.614 Volts per meter or V/m)** for pulsed RF where these exposures affect the general public; this advisory is proportionate to the evidence and in accord with prudent public health policy. A precautionary limit of 0.1 µW/cm2 should be adopted for outdoor, cumulative RF exposure. This reflects the current RF science and prudent public health response that would reasonably be set for pulsed RF (ambient) exposures where people live, work and go to school. This level of RF is experienced as whole-body exposure, and can be a chronic exposure where there is wireless coverage present for voice and data transmission for cell phones, pagers and PDAs and other sources of radiofrequency radiation. An outdoor precautionary limit of 0.1 µW/cm2 would mean an even lower exposure level inside buildings, perhaps as low as 0.01 µW/cm2. Some studies and many anecdotal reports on ill health have been reported at lower levels than this; however, for the present time, it could prevent some of the most disproportionate burdens placed on the public nearest to such installations. Although this RF target level does not preclude further rollout of WI-FI technologies, we also recommend that wired alternatives to WI-FI be implemented, particularly in schools and libraries so that children are not subjected to elevated RF levels until more is understood about possible health impacts. This recommendation should be seen as an interim precautionary limit that is intended to guide preventative actions; and more conservative limits may be needed in the future.

Broadcast facilities that chronically expose nearby residents to elevated RF levels from AM, FM and television antenna transmission are also of public health concern given the potential for very high RF exposures near these facilities (antenna farms). RF levels can be in the 10s to several 100's of µW/cm2 in residential areas within half a mile of some broadcast sites (for example, Lookout Mountain, Colorado and Awbrey Butte, Bend, Oregon). Like wireless communication facilities, RF emissions from broadcast facilities that are located in, or expose residential populations and schools to elevated levels of RF will very likely need to be re-evaluated for safety.

For emissions from wireless devices (cell phones, personal digital assistant or PDA devices, etc) there is enough evidence for increased risk of brain tumors and acoustic neuromas now to warrant intervention with respect to their use. Redesign of cell phones and PDAs could prevent direct head and eye exposure, for example, by designing new units so that they work only with a wired headset or on speakerphone mode.

These effects can reasonably be presumed to result in adverse health effects and disease with chronic and uncontrolled exposures, and children may be particularly vulnerable. The young are also largely unable to remove themselves from such environments. Second-hand radiation, like second-hand smoke is an issue of public health concern based on the evidence at hand.

V. CONCLUSIONS

- We cannot afford 'business as usual" any longer. It is time that planning for new power lines and for new homes, schools and other habitable spaces around them is done with routine provision for low-ELF environments . The business-as-usual deployment of new wireless technologies is likely to be risky and harder to change if society does not make some educated decisions about limits soon. Research must continue to define what levels of RF related to new wireless technologies are acceptable; but more research should not prevent or delay substantive changes today that might save money, lives and societal disruption tomorrow.

- New regulatory limits for ELF based on biologically relevant levels of ELF are warranted. ELF limits should be set below those exposure levels that have been linked in childhood leukemia studies to increased risk of disease, plus an additional safety factor. It is no longer acceptable to build new power lines and electrical facilities that place people in ELF environments that have been determined to be risky (at levels generally at 2 mG (0.2 µT) and above).

- While new ELF limits are being developed and implemented, a reasonable approach would be a 1 mG (0.1 µT) planning limit for habitable space adjacent to all new or upgraded power lines and a 2 mG (0.2 µT) limit for all other new construction, It is also recommended for that a 1 mG (0.1 µT) limit be established for existing habitable space for children and/or women who are pregnant . This recommendation is based on the assumption that a higher burden of protection is required for children who cannot protect themselves, and who are at risk for childhood leukemia at rates that are traditionally high enough to trigger regulatory action. This situation in particular warrants extending the 1 mG (0.1 µT) limit to existing occupied space. "Establish" in this case probably means formal public advisories from relevant health agencies.

- While it is not realistic to reconstruct all existing electrical distributions systems, in the short term; steps to reduce exposure from these existing systems need to be initiated, especially in places where children spend time, and should be encouraged.

- A precautionary limit of 0.1 (µW/cm2 (which is also 0.614 Volts per meter) should be adopted for outdoor, cumulative RF exposure. This reflects the current RF science and prudent public health response that would reasonably be set for pulsed RF (ambient) exposures where people live, work and go to school. This level of RF is experienced as whole-body exposure, and can be a chronic exposure where there is wireless coverage present for voice and data transmission for cell phones, pagers and PDAs and other sources of radiofrequency radiation. Some studies and many anecdotal reports on ill health have been reported at lower levels than this; however, for the present time, it could prevent some of the most disproportionate burdens placed on the public nearest to such installations. Although this RF target level does not preclude further rollout of WI-FI technologies, we also recommend that wired alternatives to WI-FI be implemented, particularly in schools and libraries so that children are not subjected to elevated RF levels until more is understood about possible health impacts. This recommendation should be seen as an interim precautionary limit that is intended to guide preventative actions; and more conservative limits may be needed in the future.

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SECTION 24

Key Scientific Evidence and Public Health Policy Recommendations

(Supplement 2012)

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I. INTRODUCTION

In public health and environmental policy-making, asking the right questions is a highly evolved art form. It is necessary to periodically look for ‘*not-so-early-now warnings’* from new science and medical information. At some point it becomes ‘*old news’* in the real-world process of commercializing new technologies* and is ignored. Precious time is lost if the ‘*evidence curve’* does not come quickly enough to ‘*change the rollout curve*’ and result in early enough interventions. EMF may be a highly preventable source of disease but not without early enough translation of the science into action. The time for arguing whether EMF health effects exist is over. We know they exist and that they result in human disease.

Asking the right questions and looking for proportionate responses necessarily involves make mid-course corrections guided by new evidence. This is particularly true when the consequences of doing nothing are too great to ignore – because they will affect billions of people in societies around the world. “*While there are many unanswered questions, the cost of doing nothing will result in an increasing number of people, many of them young, developing cancer*.” (Carpenter, 2010).

What questions should be asked now, to move forward on the body of evidence? How much evidence do we need to act? Do we have enough? What standard of evidence should be used to judge (purely scientific vs precautionary public health). What is a relevant biological ‘dose’? How long does a biological effect last? Are we accounting for differences among individuals or different types of cells?

Which of the studies are truly measuring chronic exposures (is a one-month or a one-year study really revealing chronic effects; if mid-length studies show no effect, does this tell us anything useful)? Why is it still considered reasonable to base safety standards on time-averaged radiofrequency exposures when the technologies today use pulsed RFR?

*Electronics, the internet, cellular telecommunications, wireless medical technologies, and wireless sensors for energy conservation, electric utilities management, transportation, education, banking and national security.

For example, the collective behavior of neurons is established through synchrony. "*Individual neurons have a time window of tens of milliseconds range for single neurons, but oscillatory coalitions of neurons can expand the effect window of synchronization from hundreds of milliseconds to many seconds*" (Buzsaki, 2006). This means the time span a bioeffect can last long enough to overlap with the next environmental provocation (pulsed RFR in this case) so that repetitive exposures may induce an unending cascade of neurological firing that eventually disrupts normal homeostasis and causes chronically abnormal function in cooperative assemblies of cells like neurons. RFR is bioactive and already classified as a Possible Human Carcinogen but the relevant RFR bursts are camouflaged and their relevant metrics are diluted away by time averaging. Why is it reasonable to use safety standards that were developed to guard against induced currents in tissue (ELF-EMF) or that heat or burn tissue (RFR)?

Briefly stated, here is what we knew in 2007.

- Bioeffects and adverse health effects of chronic exposure to low-intensity (non-thermal) non-ionizing radiation are established.
- Existing FCC and ICNIRP public safety limits are not sufficiently protective of public health.
- The World Health Organization has classified ELF-EMF as a Group 2B Possible Human Carcinogen (2001).
- New, biologically-based public exposure standards are critically needed.
- It is not in the public interest to wait.

Here is what we know in 2012. There is more evidence, over a broader range of studies. The levels of biological responses are extraordinarily low (down to the nanowatt and picowatt power density level).

New studies address fertility and reproduction, fetal and neonatal effects, cognitive and behavioral problems in children and neurological damage. There are more mobile phone base station studies with longer testing periods, much more information on genetic damage and confirmation of increased risk of brain cancers from not one or two studies, but from many studies and many authors including the World Health Organization's massive 13-country INTERPHONE STUDY (Interphone Study Group, 2010).

There are many studies reporting effects of cell phone radiation (even on standby-mode), wireless laptop exposure, cell phone use by mothers resulting in altered fetal brain development in the offspring, and more evidence that the blood-brain barrier and memory are at risk from cell phone use. There is evidence from human and animal studies that key areas of the brain are negatively affected by RFR at legal levels.

There is better understanding of the important physical and biological factors that make ELF-EMF and RFR potent disruptors of living tissues and basic metabolic processes. More and more, EMF devices are being used for medical treatments in cancer, bone and wound healing and re-tuning the nervous system. Increased depth of evidence in many threads is presented in this report by well-regarded scientists and researchers from around the world. The number of good studies has grown. The exposure levels causing effects are documented to be much lower than in the past. The epidemiological evidence is now showing risks for a variety of adverse health outcomes. All this should be taken seriously by governments, and translated quickly into more protective safety standards, and in the interim, into strong preventative actions, warnings and substitution of safer technologies and redesigned devices.

Bioeffects are clearly established and occur at very low levels of exposure to electromagnetic fields and radiofrequency radiation. Bioeffects can occur in the first few minutes at levels associated with cell and cordless phone use. Bioeffects can also occur from just minutes of exposure to mobile phone masts (cell towers), WI-FI, and wireless utility 'smart' meters that produce whole-body exposure. Chronic base station level exposures can result in illness.

Many of these bioeffects can reasonably be expected to result in adverse health effects if the exposures are prolonged or chronic. This is because they interfere with normal body processes (disrupt homeostasis), prevent the body from healing damaged DNA, produce immune system imbalances, metabolic disruption and lower resistance to disease across multiple pathways. Essential body processes can eventually be disabled by incessant external stresses (from system-wide electrophysiological interference) and lead to pervasive impairment of metabolic and reproductive functions.

What does the WHO IARC Classification of ELF-EMF and RFR as Group 2B Possible Human Carcinogens Mean?

The World Health Organization International Agency for Cancer Research (IARC) designated ELF-EMF as a Group 2B (Possible) Carcinogen in 2001. This is the kind of exposure from power lines, battery switching in cell phone devices, laptop computers and appliances. The World Health Organization specifically reaffirmed its finding that EMF is classifiable as a Group 2B Possible Human Carcinogen in 2006 in their Health Criteria Monograph #238 (WHO, 2007).

World Health Organization International Agency for Research on Cancer (IARC) Cancer Classifications

Group 1	Known Carcinogen
Group 2A	Probable Carcinogen
Group 2B	Possible Human Carcinogen
Group 3	Insufficient Information
Group 4	Not a Carcinogen

In 2011, IARC determined that scientific evidence is sufficient now to classify radiofrequency radiation as a Group 2B Possible Human Carcinogen (Baan et al, 2011). This is the kind of exposure coming from cell and cordless phones, cell towers, WI-FI, wireless laptops, electronic baby monitors and wireless ‘smart’ utility meters.

So, what does this mean? According to the classification categories, it is again clear IARC did NOT find so little clear and consistent evidence that it should support a finding of “Not A Carcinogen”. That would be the valid test that RFR is safe, as best public health experts can judge the evidence. Nor did IARC find that the evidence sufficient so as to make a stronger classification (Probably or Known Carcinogen). Rather, IARC found the evidence supports classification as a “Possible” cancer-causing agent. That is not a weak or reckless judgment made with few facts. It should be a strong warning to governments to reconsider their safety standards, particularly in light of the billions of people at potential health risk from new wireless technologies. Studies of cell and cordless phones and of wireless whole-body RFR exposures consistently show human health impacts that have become ‘epidemiologically visible’ (Sections 11 and 21).

ELF-EMF AND RFR ARE CLASSIFIED AS POSSIBLE CANCER-CAUSING AGENTS – WHY ARE GOVERNMENTS NOT ACTING?

The World Health Organization International Agency for Research on Cancer has classified wireless radiofrequency as a Possible Human Carcinogen (May, 2011). The designation applies to low-intensity RFR in general, covering all RFR-emitting devices and exposure sources (cell and cordless phones, WI-FI, wireless laptops, wireless hotspots, electronic baby monitors, wireless classroom access points, wireless antenna facilities, etc). The IARC Panel could have chosen to classify RFR as a Group 4 – Not A Carcinogen if the evidence was clear that RFR is not a cancer-causing agent. It could also have found a Group 3 designation was a good interim choice (Insufficient Evidence). IARC did neither.

II. KEY SCIENTIFIC EVIDENCE (2006- 2012)

Many thousand scientific studies over four decades have provided warnings of serious biological effects and potential health harm from EMF and RFR. About 1800 new, scientific papers published in the last five years report more bioeffects and adverse health effects of EMF and RFR, and are presented in great detail in the BioInitiative Report 2012.

These studies since 2006 give critical support to the argument that current safety standards are grossly inadequate. They cannot be protecting public health if they do not prevent harm to a variety of types of human cells, human sperm and the developing fetus *in-utero*. These are all effects reported today due to cell phone radiation exposures that are both legal and common in daily home, business and school environments. These effects are shown to occur at very low-intensity permissible levels that have become ‘typical’ for pregnant women, the fetus, the infant, the child, and for adults. Such effects are occurring at hundreds to thousands of times lower intensity exposure levels than the current FCC public safety limits allow. These exposure levels are common in the environment, but worst in close proximity to wireless devices like cell and cordless phones, 'smart' wireless utility meters, wireless routers, wireless classroom access points and laptops, to baby surveillance devices, and in the first few hundred meters of cell towers. WI-FI levels of RFR and cell phones-on-standby mode are sufficient to cause effects that, if chronic, may be damaging to the health of cellular DNA, reproductive germ cells (sperm) and the male reproductive organs.

Overall, these new studies report abnormal gene transcription (Section 5); genotoxicity and single-and double-strand DNA damage (Section 6); stress proteins because of the fractal RF-antenna like nature of DNA (Section 7); chromatin condensation and loss of DNA repair capacity in human stem cells (Sections 6 and 15); reduction in free-radical scavengers - particularly melatonin (Sections 5, 9, 13, 14, 15, 16 and 17); neurotoxicity in humans and animals (Section 9), carcinogenicity in humans (Sections 11, 12, 13, 14, 15, 16 and 17); serious impacts on human and animal sperm morphology and function (Section 18); effects on offspring behavior (Section 18, 19 and 20); and effects on brain and cranial bone development in the offspring of animals that are exposed to cell phone radiation during pregnancy (Sections 5 and 18). This is only a snapshot of the evidence presented in the BioInitiative 2014 updated report.

Many of these bioeffects are associated with disruption of normal biological functioning in the genes, and in the physiology of the nervous and cardiac systems of the body (brain, blood-brain barrier, heart, vascular system). Sleep disruption (insomnia) is a hallmark bioeffect of RFR. Hypersensitivity disorders like allergies and asthma are reported from exposure to environmental chemicals and to EMF. A pregnant woman's exposure to EMF has been linked to increased asthma and behavioral problems in the human child after *in-utero* exposure. Pregnant mice exposed to cell phone radiation give birth to baby mice with attention disorders, hyperactivity and impaired memory function, similar to effects seen in human babies as reported by Divan et al (2008).

A. Stress, Stress Proteins and DNA as a Fractal Antenna: The word stress invokes different concepts for people, but needs to be understood as a physiological response. BioInitiative author Martin Blank has described how both ELF-EMF and RFR produce stress proteins at very low exposure levels, and why this is only adaptive in the short-term. Chronic exposures that trigger stress responses (stress proteins) regardless of their environmental cause are mal-adaptive if they go on too long. Any agent (EMF, ionizing radiation, chemicals, heavy metals, etc) that continuously generates stress proteins is not adaptive, and is harmful, if it is a constant provocation.

The work of Martin Blank and Reba Goodman of Columbia University has established that stress proteins are produced by ELF-EMF and RFR at levels far below current safety standards allow. Further, they think DNA is actually a very good fractal RF-antenna which is very sensitive to low doses of EMF, and may induce the cellular processes that result in chronic 'unrelenting' stress. That daily environmental levels of ELF-EMF and RFR can and do throw the human body into stress protein response mode (out of homeostasis) is a fundamental and continuous insult. Chronic exposures can then result in chronic ill-health**.**

B. Fetal Effects and Fetal Development Studies: Effects on the developing fetus from *in-utero* exposure to cell phone radiation have been observed in both human and animal studies since 2006. Divan et al (2008) found that children born of mothers who used cell phones during pregnancy develop more behavioral problems by the time they have reached school age than children whose mothers did not use cell phones during pregnancy. The July 2008 issue of Epidemiology reports that children whose mothers used cell phones during pregnancy had 25% more emotional problems, 35% more hyperactivity, 49% more conduct problems and 34% more peer problems (Divan et al, 2008).

Aldad et al (2012) showed that cell phone radiation significantly altered fetal brain development and produced ADHD-like behavior in the offspring of pregnant mice. Exposed mice had a dose-dependent impaired glutamatergic synaptic transmission onto Layer V pyramidal neurons of the prefrontal cortex. The authors conclude the behavioral changes were the result of altered neuronal developmental programming *in utero*. Offspring mice were hyperactive and had impaired memory function and behavior problems, much like the human children in Divan et al (2008).

A new study from Greece reports altered development of the cranial bones of the mouse fetus from low intensity (0.6 to 0.9 W/kg) *in-utero* 900 MHz cell phone radiation (Fragopoulou et al, 2009). They report *“our results clearly show that even modest exposure (e.g., 6-min daily for 21 days) is sufficient to interfere with the normal mouse developmental process.”*

Other new studies by Fragopoulou et al report that brain astrocyte development followed by proteomic studies is adversely affected by DECT (cordless phone radiation) and mobile phone radiation (Fragopoulou et al, 2012); and that whole body exposure with GSM 900MHz affects spatial memory in mice (Fragopoulou et al, 2010).

FETAL BRAIN DEVELOPMENT MAY BE ALTERED

There is increasing evidence that fetal (*in-utero*) and early childhood exposures to cell phone radiation and wireless technologies in general is a risk factor for hyperactivity, learning disorders and behavioral problems in school.

Neonatal physician Carlo Bellieni of Italy found that heart rate variability is adversely affected in infants hospitalized in isolettes or incubators where ELF-EMF levels are in the 0.8 to 0.9 μT range (8 to 9 mG) (Bellieni, 2008). Infants suffer adverse changes in heart rate variability, similar to adults. He also reported that newborns cared for in the high ELF-EMF environments of isolettes have disrupted melatonin levels (Bellieni et al, 2012a).

C. Studies of Sperm: Several international laboratories have replicated studies showing adverse effects on sperm quality, motility and pathology in men who use and particularly those who wear a cell phone, PDA or pager on their belt or in a pocket (Agarwal et al, 2008; Agarwal et al, 2009; Wdowiak et al, 2007; De Iuliis et al, 2009; Fejes et al, 2005; Aitken et al, 2005; Kumar, 2012). Other studies conclude that usage of cell phones, exposure to cell phone radiation, or storage of a mobile phone close to the testes of human males affect sperm counts, motility, viability and structure (Aitken et al, 2004; Agarwal et al, 2007; Erogul et al., 2006). Animal studies have demonstrated oxidative and DNA damage, pathological changes in the testes of animals, decreased sperm mobility and viability, and other measures of deleterious damage to the male germ line

(Dasdag et al, 1999; Yan et al, 2007; Otitoloju et al, 2010; Salama et al, 2008; Behari et al, 2006; Kumar et al, 2012). There are fewer animal studies that have studied effects of cell phone radiation on female fertility parameters. Panagopoulous et al. 2012 report decreased ovarian development and size of ovaries, and premature cell death of ovarian follicles and nurse cells in *Drosophila melanogaster.* Gul et al (2009) report rats exposed to stand-by level RFR (phones on but not transmitting calls) caused decrease in the number of ovarian follicles in pups born to these exposed dams. Magras and Xenos (1997) reported irreversible infertility in mice after five (5) generations of exposure to RFR at cell phone tower exposure levels of less than one microwatt per centimeter squared (μW/cm2).

Agarwal et al (2009) evaluated the effect of cell phone radiation during talk mode on human sperm samples. The authors found *"radiofrequency electromagnetic waves emitted from cell phones may lead to oxidative stress in human semen. We speculate that keeping the cell phone in a trouser pocket in talk mode may negatively affect spermatozoa and impair male fertility."*

Aitken et al (2005) studied the effect of 900 MHz cell phone radiation on mice (7 days, 12-hr per day at 0.09 W/kg). The authors found statistically significant damage to the mitochondrial genome of epididymal spermatozoa ($p<0.05$).

Avendano et al, 2012 provided evidence that a 4-hr exposure to WI-FI at exceeding low levels (0.5-1.0 μW/cm2) near a laptop computer caused decreased sperm viability and DNA fragmentation in human sperm samples. Avendado says "(*T)o our knowledge, this is the first study to evaluate the direct impact of a laptop use on human spermatozoa. Ex vivo exposure of human spermatozoa to a wireless internet-connected laptop decreased motility and induced DNA fragmentation by a nonthermal effect. We speculate that keeping a laptop connected wirelessly to the internet on the lap near the testes may result in decreased male fertility."*

De Iuliis et al (2009) reported that *"RF-EMR in both the power density and frequency range of mobile phones enhances mitochondrial reactive oxygen species generation by human spermatozoa, decreasing the motility and vitality of these cells* *while stimulating DNA base adduct formation, and ultimately DNA fragmentation.*" They warned their findings "*have clear implications for the safety of extensive mobile phone use by males of reproductive age, potentially affecting both their fertility and the health and wellbeing of their offspring*" based on damage from a 6-hr exposure to 1800 MHz cell phone radiation in human sperm cells. This 6-hr exposure caused reduced sperm motility and viability and caused a significant increase in reactive oxygen species (free radicals that are associated with oxidative damage to DNA), and the effects were worse with more exposure (a significant dose-response was observed). Atasoy (2012) also questioned the safety of 2400 MHz exposure to those of reproductive age. This study reports that WI-FI internet access devices can damage DNA and reduce DNA repair when the exposures are very low(exposure level of 0.091 W/kg) and chronic; damage can occur even at levels that comply with 802.11 g WI-FI public safety limits.

Behari et al (2006) reported that chronic exposure of rats to cell phone radiation caused double-strand DNA breaks in sperm cells (35 days, 2-hr per day). This study also showed that the mobile radiation exposure at 900 MHz (at 0.9 W/kg) and at 2.45 GHz (at 0.1 W/kg) caused a statistically significant decrease in sperm count and the weight of testes.

Otitoloju et al., (2010) graphically describe sperm head abnormalities in mice exposed for six months to base-station level RF/MW at 70 to 100 nanowatts/cm2 (0.07 – 0.1 μW/cm2). Only 2% of controls but a stunning 39% to 46% of exposed mice had damaged sperm.

> "*The major abnormalities observed were knobbed hook, pin-head and banana-shaped sperm head. The occurrence of sperm head abnormalities was also found to be dose dependent. The implications of the observed increased occurrence of sperm head abnormalities on the reproductive health of humans living in close proximity to GSM base stations were discussed.*"

These studies taken together should provide a strong warning that 'normal' use of a cell phone presents risks that warrant strong preventative actions to protect the integrity of the human genome from de novo mutations and loss of fertility across entire male populations of cell phone users. Further, even the much lower exposure levels associated with mobile phone base station (cell tower) RFR levels are deleterious over time.

HUMAN SPERM AND THEIR DNA ARE DAMAGED

Human sperm are damaged by cell phone radiation at very low intensities (0.00034 – 0.07 μW/cm2). There is a veritable flood of new studies reporting sperm damage in humans and animals, leading to substantial concerns for fertility, reproduction and health of the offspring (unrepaired de novo mutations in sperm). Exposure levels are similar to those resulting from wearing a cell phone on the belt, or in the pants pocket, or using a wireless laptop computer on the lap. Sperm lack the ability to repair DNA damage.

D. Human Stem Cell Studies: Markova et al (2010) reported that 915 MHz microwave exposure significantly affects human stem cells. They found that very low-intensity microwave radiation from mobile phones can inhibit DNA repair processes in human stem cells. By placing a mobile phone at one meter distance from human stem cells in petri dishes (SAR = 0.037 W/Kg), they found a significant reduction in 53BP1 foci. These foci are a measure of DNA repair in cells with double strand DNA damage. The damage was greater to stem cells (derived from adipose tissue in humans) than in fibroblasts. Stem cells did not repair over time - and the damage was done within one hour of microwave exposure. Fibroblasts were similarly affected (inhibited 53BP1 foci) but repaired over time. The effects are carrier-frequency dependent. The effects occurred with GSM exposure at 915 MHz, but not at 905 MHz. The failure of DNA repair also occurred at the mobile phone UTMS carrier frequency of 1947 MHz. Analysis of the 53BP1 foci is a sensitive technique to measure double-strand DNA breaks in both unexposed cells and in cells exposed to cytotoxic agents. In the authors' words, *"this represents a direct mechanistic link to epidemiological data showing an association of MW exposure with increased cancer risk*." The data obtained from human stem cells is of "*utmost relevance for assessment of possible health risks of MW exposure from mobile phones."* Most, if not all adult tissues and organs including blood, skin and brain contain stem cells. Therefore, "*stem cells like blood cells and fibroblasts are always subjected to exposure from mobile phones*." With respect to children, because "*almost all organs and tissues possess stem cells and stem cells are more active in children, the possible relationship of chronic MW exposure and various types of tumors and leukemia especially in children should be investigated.”*

Czyz et al (2004) reported that GSM cell phone exposure affected gene expression levels in embryonic stem cells (p53-deficient); and significantly increased heat shock protein HSP 70 production.

HUMAN STEM CELL DNA DOES NOT ADAPT OR REPAIR

Human adipose tissue stem cells lack the ability to repair DNA damage caused by chronic exposure to non-thermal microwaves. Damage to DNA in some other cells may be incompletely repaired.

E. Mobile Phone Base Station (Cell Tower) Studies

Human Studies: Hutter et al (2006) reported that short-term exposure to GSM cell phone radiation resulted in complaints of headache, neurological problems, sleep and concentration problems in adults with 0.01 - 0.05 μW/cm2 exposure levels. Kundi and Hutter (2009) reviewed human effects in fourteen (14) mobile phone base station studies and reported *"(F)rom available evidence it is impossible to delineate a threshold below which no effect occurs, however, given the fact that studies reporting low exposure were invariably negative it is suggested that power densities around 0.5–1 mW/m2 [*0.05 – 0.1 uW/cm2*] must be exceeded in order to observe an effect."*

Buchner and Eger (2012) conducted an eighteen (18) month study to assess changes in stress hormones in 60 persons exposed before and after a mobile phone base station went into operation in the Rimbach village in Germany. The study showed that chronic exposure to base station RF (whole-body) at 0.006 - 0.01 μW/cm2 in humans had significant impacts on stress hormones over time. In the beginning months, adrenaline levels first increased in a dose-dependent fashion according to exposure level ($p < 0.002$) and then decreased below normal levels ($p < 0.005$). Both the average as well as the median adrenaline values increased after the activation of the transmitter and decreased again after one year with exposure levels >0.006 μW/cm2. Chronically ill subjects and children showed especially strong responses; except for some "outliers," no effect was observed in healthy adults (Buchner and Eger, 2012). For dopamine, inverse effects to those for adrenaline and noradrenaline were observed. The median dopamine levels decreased from 199 to 115 µg/g creatinine between January and July 2004. The fact that the dopamine levels of the study subjects decreased during this period is highly significant ($p<0.0002$). Thereafter, the median increased again: In January 2005, it was at 131 µg/g creatinine, in July of 2005. This increase is also significant between July 2004 and July 2005 ($p<0.05$).

Buchner (2012) indicates that the RFR transmitter induced changes in stress hormones that follow the classic stress syndrome of adaptation, then exhaustion established by Hans Seyle in the 1950's. *"After the stages of alarm and resistance, the last stage of exhaustion sets in. The parameters investigated in the Rimbach study follow this pattern"*.

A long-term 6-yr study assessed the role of exposure to radio frequency radiation (RFR) emitted either from mobiles or base stations and its relations with human's hormone profiles. The study revealed significant RFR effects on pituitary–adrenal axis, resulting in reduction of ACTH, cortisol, thyroid hormones, prolactin in young females, and testosterone levels in males (Eskander et al, 2012). But no direct measurements of RFR power density levels were made, only categories of distance from transmitter.

Oberfeld et al (2004) reported that populations exposed to base stations transmitting cell phone frequencies had more fatigue, depressive tendency, sleeping disorders, concentration difficulties, and cardio-vascular problems reported with exposure to GSM 900/1800 MHz cell phone signal.

Navarro et al (2003) reported that exposure levels of 0.01 - 0.11 µW/cm2 resulted in fatigue, headaches, sleeping problems in populations around mobile phone base stations.

Thomas et al (2008) reported an increase in adult complaints of headaches and concentration difficulties with short-term cell phone use at 0.005 to 0.04 µW/cm2 exposure levels.

Heinrich et al (2010) reported that children and adolescents (8-17 years old) with short-term exposure to base-station level RFR experienced headache, irritation, and concentration difficulties in school. RFR levels were 0.003 - 0.02 µW/cm2.

Thomas et al (2010) reported that RFR levels of 0.003 - 0.02 μW/cm2 resulted in conduct and behavioral problems in children and adolescents (8-17 years old) exposed to short-term cell phone radiation in school.

Mohler et al (2010) reported that adults exposed to 0.005 μW/cm2 cell phone radiation (base-station exposure levels) had sleep disturbances with chronic exposure, but this effect was not significantly increased across the entire population.

Human Studies at Base Station Exposure Levels (Cell Towers)

At least five new cell tower studies with base-station level RFR at levels ranging from 0.003 μW/cm2 to 0.05 uW/cm2 published since 2007 report headaches, concentration difficulties and behavioral problems in children and adolescents; and sleep disturbances, headaches and concentration problems in adults. This is highly consistent with studies done prior to 2007, but the 'effect levels' are significantly lower (dropping from the microwatt to the nanowatt range per square centimeter).

Public safety standards are 1,000 – 10,000 or more times higher than levels now commonly reported in mobile phone base station studies to cause bioeffects.

Sperm studies are showing DNA damage, impaired sperm quality, motility and viability from cell phones on standby mode and wireless laptop use at exposures of 0.00034 μW/cm2 to 0.07 μW/cm2. Several studies report sperm damage effects at 'standby model' cell phone emission levels, which are in the low nanowatt to picowatt per square centimeter range.

F. Electrohypersensitivity (EHS) Studies: McCarty et al (2011) studied electrohypersensitivity in a patient (a female physician). The patient was unable to detect the presence or absence of EMF exposure, largely ruling out the possibility of bias. In multiple trials with the fields either on or not on, the subject experienced and reported temporal pain, feeling of usease, skipped heartbeats, muscle twitches and/or strong headache when the pulsed field (100 ms, duration at 10 Hz) was on, but no or mild symptoms when it was off. Symptoms from continuous fields were less severe than with pulsed fields. The differences between field on and sham exposure were significant at the $p < 0.05$ level. The authors conclude that electromagnetic hypersensitivity is a neurological syndrome, and statistically reliable somatic reactions could be provoked in this patient by exposure to 60-Hz electric fields at 300 volts per meter (V/m). They conclude *"EMF hypersensitivity can occur as a bona fide environmentally inducible neurological syndrome*." In their response to a letter to the editor of the journal, the authors say: *"(W)e followed an empirical approach and demonstrated a cause-and-effect relationship ($p < 0.05$) under conditions that permitted us to infer the existence of electromagnetic hypersensitivity (EHS), a novel neurological syndrome*." (Marino et al., 2012)

Further, the authors explain the significance of detecting EHS effects by non-linear methods.

> *"The important issue at this point is not whether EMF can produce symptoms (we empirically demonstrated that it can) but rather why this effect historically has been difficult to detect. It occurred to us that EHS has remained elusive because of the way it was studied. The experiments designed to detect EHS had been based on the assumption that if it existed, it was a linear phenomenon, whereas EHS is actually a nonlinear phenomenon." "Our study was designed to detect whether EHS was a linear or nonlinear phenomenon, and we were successful in showing a link between acute EMF exposure and somatic responses ($p < 0.05$). This finding – taken together with the unfailingly negative results of the linear studies – is good evidence that EHS is a nonlinear phenomenon, as we suspected."*

With the exception of the McCarty study there have not been clear demonstrations in controlled circumstances showing that persons reporting to be electrophypersensitive can distinguish whether or not RFR is being applied. There are, however, multiple reports of symptoms experienced by individuals exposed to EMFs in uncontrolled circumstances.

A. Johansson et al (2010) studied symptoms, personality traits and stress in people with mobile phone-related symptoms and electromagnetic hypersensitivity. They reported there is support for a difference between people with symptoms related to specific EMF sources and people with general EHS. The symptoms are anxiety, depression, somatization, exhaustion and stress. The EHS group reported more neurasthenic symptoms.

Two publications on electrohypersensitivity by O. Johansson (2007, 2009) provide an extensive overview of the relevant literature on electrohypersensitivity. Both publications document symptoms and conditions giving rise to increased sensitivity to ELF-EMF and RFR. The need for new, biologically-based public exposure standards is recommended in both publications, in order to address electrohypersensitivity.

Landgrebe et al (2007) reported that their study of electrosensitive patients showed participants had a reduced intracortical facilitation as compared to two control groups. The EHS group of patients showed altered central nervous system function. In a follow-up study, the authors reported that EHS patients but not controls "*demonstrated significant cognitive and neurobiological alterations pointing to a higher genuine individual vulnerability of electromagnetic hypersensitive patients." (Landgrebe et al, 2008).*

The team of Sandstrom, Hansson Mild and Lyskov produced numerous papers between 1994 and 2003 involving people who are electrosensitive (Lyskov et al, 1995; Lyskov et al, 1998; Sandstrom et al, 1994; Sandstrom et al, 1995; Sandstrom et al, 1997; Sandstrom et al, 2003). Sandstrom et al (2003) presented evidence that heart rate variability is impaired in people with electrical hypersensitivity and showed a dysbalance of the autonomic nervous system. *"EHS patients had a disturbed pattern of circadian rhythms of HRF and showed a relatively 'flat' representation of hourly-recorded spectral power of the HF component of HRV".* This research team also found that *"EHS patients have a dysbalance of the autonomic nervous system (ANS) regulation with a trend to hyper-sympathotonia, as measured by heart rate (HR) and electrodermal activity, and a hyperreactivity to different external physical factors, as measured by brain evoked potentials and sympathetic skin responses to visual and audio stimulation*." (Lyskov et al, 2001 a,b; Sandstrom et al, 1997). The reports referenced above provide evidence that persons who report being electrosensitive differ from others in having some abnormalities in the autonomic nervous system, reflected in measures such as heart rate variability. At present it remains unclear whether EHS is actually caused by RF/EMF exposure, or rather is a self-identifying syndrome of excessive responsiveness to a variety of stimuli. But given the relatively high percentage of persons reported to be electrosensitive (5% of the general population of Switzerland according to Schreier et al, 2006), with some being severely disabled as a consequence, it is critical that there be more study of this syndrome.

Tuengler and von Klitzing et al (2012) reported EHS people that were tested showed significant changes in regulation of the autonomic nervous system, including changes in capillary blood flow (microcirculation), heart rate variability, and electric skin potentials. The continuous detection of capillary blood flow is an important tool in analyzing the capacity of the autonomic nervous system. In EHS patients, von Klitzing finds that intestinal motility may also be disregulated and show no activity at all for some time after exposure.

G. Effects on the Blood-brain Barrier (BBB): The Lund University (Sweden) team of Leif Salford, Bertil Persson and Henrietta Nittby has done pioneering work on effects of very low level RFR on the human brain's protective lining – the barrier that protects the brain from large molecules and toxins that are in the blood.

THE BLOOD-BRAIN BARRIER IS AT RISK

The BBB is a protective barrier that prevents the flow of toxins into sensitive brain tissue. Increased permeability of the BBB caused by cell phone RFR may result in neuronal damage. Many research studies show that very low intensity exposures to RFR can affect the blood-brain barrier (BBB) (mostly animal studies). Summing up the research, it is more probable than unlikely that non-thermal EMF from cell phones and base stations do have effects upon biology. A single 2-hr exposure to cell phone radiation can result in increased leakage of the BBB, and 50 days after exposure, neuronal damage can be seen, and at the later time point also albumin leakage is demonstrated. *The levels of RFR needed to affect the BBB have been shown to be as low as 0.001 W/kg, or less than holding a mobile phone at arm's length. The US FCC standard is 1.6 W/kg; the ICNIRP standard is 2 W/kg of energy (SAR) into brain tissue from cell/cordless phone use. Thus, BBB effects occur at about 1000 times lower RFR exposure levels than the US and ICNIRP limits allow.* *(Salford, 2012)*

The consequence to modern life is that cell and cordless phone use may cause a pathological leakage of the BBB with very short use periods, and the damage may be long-lasting. Harmful substances may enter the brain. If the damage is ongoing (if cell and cordless phone use continues to occur over months and years), the potential for harmful effects increases. There is already 'epidemiologically visible' evidence of increased brain cancer risk in humans (Section 11).

Volkow et al (2011a, b) reported increased glucose metabolism in the brain with cell phone use in humans. This important investigation of 47 human subjects used a randomized crossover design and labeled fluorodeoxyglucose to measure the metabolisms of the brain when the cell phone was activated but muted for 50 minutes as compared to not being activated. *"Our study showed that cell phone activation was associated with metabolic increases in brain regions closest to the antenna and that the increases showed a negative linear correlation with distance from the antenna. While the effect was small, the negative correlation of the effect with distance was statistically significant (R = –0.91; P <.001).* This study is particularly important in that it demonstrates definitively that an active cell phone, placed on the ear as one would normally be used, alters brain metabolic activity, but only in the region close to the cell phone.

H. Brain Cancer Studies: The Orebro University (Sweden) team led by Lennart Hardell, MD, an oncologist and medical researcher, has produced an extraordinary body of work on environmental toxins of several kinds, including the effects of radiofrequency/microwave radiation and cancer. Their 2012 work concludes:

> *"Based on epidemiological studies there is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of mobile phones and cordless phones. The evidence comes mainly from two study centres, the Hardell group in Sweden and the Interphone Study Group. No consistent pattern of an increased risk is seen for meningioma. A systematic bias in the studies that explains the results would also have been the case for meningioma. The different risk pattern for tumor type strengthens the findings regarding glioma and acoustic neuroma. Meta-analyses of the Hardell group and Interphone studies show an increased risk for glioma and acoustic neuroma. Supportive evidence comes also from anatomical localisation of the tumor to the most exposed area of the brain, cumulative exposure in hours and latency time that all add to the biological relevance of an increased risk. In addition risk calculations based on estimated absorbed dose give strength to the findings.*

In summary:

- *There is reasonable basis to conclude that RF-EMFs are bioactive and have a potential to cause health impacts.*

- *There is a consistent pattern of increased risk for glioma and acoustic neuroma associated with use of wireless phones (mobile phones and cordless phones) mainly based on results from case-control studies from the Hardell group and Interphone Final Study results.*
- *Epidemiological evidence gives that RF-EMF should be classified as a human carcinogen.*
- *Based on our own research and review of other evidence the existing FCC/IEE and ICNIRP public safety limits and reference levels are not adequate to protect public health.*
- *New public health standards and limits are needed.* (Hardell et al, 2012)

I**. Genetic Damage (Genotoxicity Studies):** There are at least several hundred published papers that report EMF affects cellular oxidative processes (oxidative damage). Increased free radical activity and changes in enzymes involved in cellular oxidative processes are the most consistent effects observed in cells and animals after EMF exposure. Aging may make an individual more susceptible to the detrimental effects of ELF EMF from oxidative damage, since anti-oxidants may decline with age. Clearly, the preponderance of genetic studies report DNA damage and failure to repair DNA damage.

Eighty six (86) new papers on genotoxic effects of RFR published between 2007 and mid-2012 are profiled. Of these, 54 (63%) showed effects and 32 (37%) showed no effects (Lai, 2012)

Forty three (43) new ELF-EMF papers and two static magnetic field papers that report on genotoxic effects of ELF-EMF published between 2007 and mid-2012 are profiled. Of these, 35 (81%) show effects and 8 (19%) show no effect (Lai, 2012).

J. Nervous System Damage: Factors that act directly or indirectly on the nervous system can cause morphological, chemical, or electrical changes in the nervous system that can lead to neurological effects. Both RF and ELF EMF affect neurological functions and behavior in animals and humans.

One hundred fifty five (155) new papers that report on neurological effects of RFR published between 2007 and mid-2012 are profiled. Of these, 98 (63%) showed effects and 57 (37%) showed no effects.

Sixty nine (69) new ELF-EMF papers (including two static field papers) that report on genotoxic effects of ELF-EMF published between 2007 and mid-2012 are profiled. Of these, 64 (93%) show effects and 5 (7%) show no effect. (Lai, 2012)

L. Children are More Vulnerable: Many studies demonstrate that children are more sensitive to environmental toxins of various kinds (Barouki et al, 2012; Preston, 2004; WHO, 2002; Gee, 2009; Sly and Carpenter, 2012).

> The Presidential Cancer Panel (2010) found that children '*are at special risk due to their smaller body mass and rapid physical development, both of which magnify their vulnerability to known carcinogens, including radiation.*'

> The American Academy of Pediatrics, in a letter to Congressman Dennis Kucinich dated 12 December 2012 states "*Children are disproportionately affected by environmental exposures, including cell phone radiation. The differences in bone density and the amount of fluid in a child's brain compared to an adult's brain could allow children to absorb greater quantities of RF energy deeper into their brains than adults. It is essential that any new standards for cell phones or other wireless devices be based on protecting the youngest and most vulnerable populations to ensure thay are safeguarded through their lifetimes.*"

II. ISSUES AND ANSWERS IN THE EMF DEBATE

Much of the emphasis in the 2007 Bioinititative Report focused on cancer, which is still the best documented disease of concern from exposure to EMF/RF. The evidence that exposure to EMF/RF increases the risk of cancer has only gotten significantly stronger since then, and we have a better, albeit still incomplete, understanding of the mechanisms involved. However, in terms of threshold exposures that result in human disease, new research on male reproduction and neurobehavioral alterations provide evidence for harm at even lower exposure levels. RFR has been shown in this Report to act as an external synchronizer of neural activity, capable of disrupting sleep, circadian rhythms, diurnal hormone fluctuations, brain wave activity and heart rate variability by exposure to artificial electromagnetic signals (as opposed to natural evolutionary frequencies) and to do so at exceedingly low intensities.

Much of the debate over the body of EMF science ignores simple questions that would help to discriminate among studies with apparently conflicting results. Section 15 by Dr. Belyaev is helpful in identifying key factors which must be known and controlled for in experiments (biological variables and physical parameters include bandwidth, frequency, modulation, polarization, intermittence and coherence time of exposure, static magnetic field, electromagnetic stray fields, sex, age, individual traits, and cell density during exposure). Dr. Andrew Marino emphasizes that detection of EMF/RFR effects require investigation of non-linear phenomena, a critical difference that if ignored, may miss important biological effects (Marino, 2012).

A unifying hypothesis for a plausible biological mechanism to account for very weak field EMF bioeffects other than cancer may lie with weak field interactions of pulsed RFR and ELF-modulated RFR as disrupters of synchronized neural activity. Electrical rhythms in our brains can be influenced by external signals. This is consistent with established weak field effects on coupled biological oscillators in living tissues. Biological systems of the heart, brain and gut are dependent on the cooperative actions of cells that function according to principles of non-linear, coupled biological oscillations for their synchrony, and are dependent on exquisitely timed cues from the environment at vanishingly small levels (Buzsaki, 2006; Strogatz, 2003). The key to synchronization is the joint actions of cells that co-operate electrically - linking populations of biological oscillators that couple together in large arrays and synchronize spontaneously according to the mathematics described for Josephson junctions (Brian Josephson, the 1993 Nobel prize winner for this concept). This concept has been professionally presented in journal articles and also popularized in print by Prof. Steven Strogatz, a mathematician at Cornell University who has written about ‘sync’ as a fundamental organizing principle for biological systems (Strogatz, 2001; 2003).

> *“Organisms are biochemically dynamic. They are continuously subjected to time-varying conditions in the form of both extrinsic driving from the environment and intrinsic rhythms generated by specialized cellular clocks within the organism itself. Relevant examples of the latter are the cardiac pacemaker located at the sinoatrial node in mammalian hearts and the circadian clock residing at the suprachiasmatic nuclei in mammalian brains. These rhythm generators are composed of thousands of clock cells that are intrinsically diverse but nevertheless manage to function in a coherent oscillatory state. This is the case, for instance, of the circadian oscillations exhibited by the suprachiasmatic nuclei, the period of which is known to be determined by the mean period of the individual neurons making up the circadian clock. The mechanisms by which this collective behavior arises remain to be understood.”(Strogatz, 2003)*

Synchronous biological oscillations in cells (pacemaker cells) can be disrupted by artificial, exogenous environmental signals, resulting in desynchronization of neural activity that regulates critical functions (including metabolism) in the brain, gut and heart and circadian rhythms governing sleep and hormone cycles (Strogatz, 1987). The brain contains a population of oscillators with distributed natural frequencies, which pull one another into synchrony (the circadian pacemaker cells). Strogatz has addressed the unifying mathematics of biological cycles and external factors disrupt these cycles. Buzsaki (2006) says "*rhythms can be altered by a wide variety of agents and that these perturbations must seriously alter brain performance. Rhythms are a robust phenomenon.*"

The heart's natural pacemaker center is the sinoatrial node, a cluster of about 10,000 cells that generate electrical rhythm that commands the rest of the heart to beat. Diseases related to disruption of that synchronization include epilepsy, chronic insomnia, and cardiac arrhythmias (Strogatz, 2003). Some EMF diseases are those where desynchronization of neural activity results in physiological changes that, if chronic, result in chronically disrupted homeostasis, and eventually ill-health and chronic diseases. Such a future burdens health care systems in an irreversible way.

The late Dr. Ross Adey in his last publication in Bioelectromagnetic Medicine (P. Roche and M. Markov, eds. 2004) concluded:

> "*There are major unanswered questions about possible health risks that may arise from exposures to various man-made electromagnetic fields where these human exposures are intermittent, recurrent, and may extend over a significant portion of the lifetime of the individual.*"
>
> *"Epidemiological studies have evaluated ELF and radiofrequency fields as possible risk factors for human health, with historical evidence relating rising risks of such factors as progressive rural electrification, and more recently, to methods of electrical power distribution and utilization in commercial buildings. Appropriate models describing these bioeffects are based in nonequilibrium thermodynamics, with nonlinear electrodynamics as an integral feature. Heating models, based in equilibrium thermodynamics, fail to explain an impressive new frontier of much greater significance. Though incompletely understood, tissue free radical interactions with magnetic fields may extend to zero field levels."*

Our society appears determined to make everything wireless, and the consequence is to increase cumulative exposure to RFR. Many homes and almost every Starbucks or McDonalds has WiFi. Smart phones, tablets, video iPods and other wireless devices are even given to children as playthings. The result is a significant increase in cumulative RFR exposure of the whole population, but particularly of those who have and use wireless devices for prolonged periods of time. No national or international standard of RFR exposure considers cumulative effects, all being developed to avoid local tissue heating from acute exposures.

The issues around exposure of children to RFR is of critical importance. There is overwhelming evidence that children are more vulnerable than adults to many different exposures (Sly and Carpenter, 2012), including RFR, and that the diseases of greatest concern are cancer and effects on neurodevelopment. Yet parents place RFR baby monitors in cribs, provide very young children with wireless toys, and give cell phones to young children, usually without any knowledge of the potential dangers. A growing concern is the movement to make all student computer laboratories in schools wireless. A wired computer laboratory will not increase RFR exposure, and will provide safe access to the internet.

An urgent example for the need to address the lack of adequate public protection from inadequate safety standards for pulsed RFR exposures is the rapid, global rollout of wireless utility meters ('smart' meters for electricity, gas and water meters). Current safety standard calculations that rely on time-averaging of RFR almost entirely dilute out the power density of RFR levels that are delivered in millisecond bursts, but occur at intervals of every second, or multiple times per second when in use within a wireless mesh network. Said differently, the RFR power density levels are usually legal. While there have been no long term studies of adverse effects of smart meters on human health (primarily because they are so new), there are increasing reports from electrosensitive individuals of harm. Added together, these RFR pulses that now appear to be a highly bioactive agent but are essentially erased or made energetically invisible by time-averaging the pulses as current FCC safety rules mandate.

The wireless meters transmit RF signals like a mini-cell tower antennas in the cell phone radiation frequencies. Currently, they are being deployed in the US and are on the drawing boards around the world including many European countries. The 'smart meter' infrastructure represents the largest single commercial saturation of living space with pulsed RFR yet rolled out by industry. This program places a wireless device (like a mini-mobile phone base station) on the wall, replacing the electromechanical (spinning dial) meter. They will be installed on every home and classroom (every building with an electric meter). Utilities from California to Maine have installed tens of millions already, despite health concerns of experts who already are seeing thousands of health complaints. The wireless meters produce spikes of pulsed radiofrequency radiation on a continuous basis (24/7), and in typical operation, will saturate living space at levels that can be much higher than already reported to cause bioeffects and adverse health effects for some people. These meters, depending on where they are placed relative to occupied space in the home or classroom, can produce RFR exposure levels similar to that within the first 100 feet to 600 feet of a mobile phone base station (cell tower). In the not-so-distant future the plan is to have a wireless device implanted in every household appliance, which will communicate with the smart meter whenever electricity is being used. This will likely make the kitchen a major source of exposure to RFR.

The cumulative RFR burden within any community is largely unknown. Both involuntary sources (like cell towers, smart meters and second-hand radiation from the use of wireless devices by others) plus voluntary exposures from ones' personal use of cell and cordless phones, wireless routers, electronic baby surveillance monitors, wireless security systems, wireless hearing aids, and wireless medical devices like implanted insulin pumps all add up. No one is tallying up the combined exposure levels. Billions of new RFR transmitters from a global smart meter rollout will significantly add to the existing RFR body-burden of pulsed RFR for millions of people. The health concerns are the same as with all other sources of EMF/RFR. Cancer is a serious adverse effect, but damage to male reproduction and central nervous system effects may results from even lower levels of exposure. The work by Strogatz (2001, 2003) and Buzsaki (2006) on weak-field effects on non-linear biological oscillators (brain waves and synchronization of neural activities that regulate body processes) is directly relevant to an understanding of the profound biological disruptions and health symptoms that continued exposures of pulsed RFR may produce.

The Commons of the Air

Turning to questions of social equity and the individuals' choice not to be exposed to harmful levels of environmental toxins, there has been little inclusion of the public in discussions of wireless radiofrequency exposure. Wireless technologies have become infused in daily habits of billions of people; often choices for wired equivalents are lacking (or those that exist are disappearing). Involuntary exposure to EMF and RFR is becoming more the norm, even where it runs counter to individual choice (second-hand radiation, like second-hand smoke is difficult to avoid).

> *"Wireless technologies drive electromagnetic energy through our air, into and through virtually all indoor and outdoor living environments. The protective air cushion around our planet holds breathable air, buffers us from space radiation, and supports and sustains life in tandem with the natural electromagnetic signature of the earth itself. We are changing this 'commons of the air' in major ways. Wireless signals from broadcast and communications technologies are crowding out and overpowering the natural background. The 'commons of the air' is being altered in unprecedented ways that have enormous consequences for life on earth."(Sage, 2010).*

The rush to 'buy the airwaves' and to market them for commercial purposes is loading '*the commons of the air*' with unsustainable levels of exposure (Sage, 2010). Commercial markets for wireless spectrum successfully lobby government regulators to allocate even more spectrum, once the existing frequencies are allocated. Sage (2010) asks:

> *"Who owns the 'commons of the air'? Who should be allowed to pollute it? What are the limits? On what basis should carrying capacity be defined? Who defines the limits? Do these limits conserve the resource for the future? Do they protect public health and welfare, and the health and well-being of other living things on earth? Who bears the burden of proof of safety or of harm? How should the 'new commons' be managed for the greater good? Do we know enough to act responsibly? Who decides? When should limits be placed on utilization?"*

With no regard to cumulative harm, this commercial rush to buy up wireless spectrum territorial rights has vast implications for public health and well-being. Environmental protections afforded to other natural resources under the National Environmental Policy Act have been ignored. The cumulative impacts and irretrievable commitments on humans, wildlife, and natural resources have never been assessed.

> *"Societies must now define carrying capacity for chronic electromagnetic and wireless exposures. Taking into account the large individual variability to withstand it, new limits must conserve and sustain the 'commons of the air' so that is sustainable for all—and this includes sensitive populations, the young, the elderly, and those with existing sensitivity. Some countries of the world already have surpassed sustainable wireless exposure levels as demonstrated by significant percentages that have already become electrosensitive." (Sage, 2010)*

Homeostasis and Human Health Rights

Chronic exposure to low-intensity RFR and to ELF-modulated RFR at today's environmental levels in many cities will exceed thresholds for increased risk of many diseases and causes of death (Sage and Huttunen, 2012). RFR exposures in daily life alter homeostasis in human beings. These exposures can alter and damage genes, trigger epigenetic changes to gene expression and cause de novo mutations that prevent genetic recovery and healing mechanisms. These exposures may interfere with normal cardiac and brain function; alter circadian rhythms that regulate sleep, healing, and hormone balance ; impair short-term memory, concentration, learning and behavior; provoke aberrant immune, allergic and inflammatory responses in tissues; alter brain metabolism; increase risks for reproductive failure (damage sperm and increase miscarriage risk); and cause cells to produce stress proteins. Exposures now common in home and school environments are likely to be physiologically addictive and the effects are particularly serious in the young (Sage and Huttunen, 2012). This declaration of human health rights below (Sage and Huttunen, 2012) is based on specific reference to health impacts of EMF and RFR that are reasonably well established to occur (Sage and Carpenter, 2009).

Human Health Rights Declaration

Fundamental Human Health Rights (*Sage and Huttunen, 2012*)

The right to homeostasis in our own bodies.
The right to normal central nervous system function.
The right to natural environmental cues that synchronize our circadian rhythms.
The right to sleep.
The right to heal.
The right to hear.
The right to reproduce.
The right to learn and retain memories.
The right to an intact genome.

If even one of these rights is compromised – placed at risk from involuntary wireless exposures in daily life, it is a breach of human health rights. When many of these human health rights are compromised without the consent of the individual, then the deployment of wireless technologies should be halted and existing exposures reduced or eliminated, in accord with the scientific and public health findings on chronic exposure to low-intensity radiofrequency radiation, and other forms of potentially harmful electromagnetic fields (Sage and Huttunen, 2012)

V. CONCLUSIONS FOR PRUDENT PUBLIC HEALTH PLANNING

Methodology and Approach for Precautionary Action Limits

In 2007, the BioInititive Report chapter on Key Scientific Evidence and Public Health Policy Implications, proposed a specific, interim radiofrequency radiation target level of 0.1 μW/cm2 for cumulative, outdoor RFR exposure (for AM, FM, TV and wireless). It was based on best-available scientific studies to that date. There were few studies prior to 2006 that reported effects at less than 0.1 to 1 μW/cm2 chronic RFR exposures.

In 2009, the journal Pathophysiology produced many peer-reviewed articles in a special two-volume edition on EMF (both ELF-EMF and RFR) essentially publishing the contents of the BioInitiative Report and updating some information. One of these 2009 Pathophysiology papers presented a review of mobile phone base station studies (Kundi and Hutter, 2009). It concluded that the overall studies did not detect effects (headache, fatigue, tinnitus, concentration difficulties, sleep disruption, etc) at levels of RFR exposure below 0.05 to 0.1 μW/cm2.

New base station-level RFR studies are available in 2012 that can be analyzed to determine if new (and lower) RFR recommendations are warranted. The approach in this chapter relies on "lowest levels at which effects are not seen" akin to the “no observed effect level (NOEL)” used for chemical exposures, as a sufficient basis to establish scientific benchmarks for harm (or alternately, the lowest observed effects level of exposure). It is the province of the science and public health evaluation we do here to report the evidence regardless of what political or strategic complications it may create. An objective presentation of what the studies reveal for ‘effects levels’ is our goal; not to pre-judge or dilute the evidence because it may present strategic or political hurdles to achieve consensus on policy and regulatory changes. What this report does not intend to do is take into account“how could we do this” or “what would it mean”. The purpose is to lay out the science, and make some defensible reductions for factors that studies cannot or do not yet test for, and compensate with deductions for them (safety margins). As interim targets for precautionary action, they will serve as guides for decision-makers who will take up the issues of health, the quality of the future gene pool, social equity and cost.

There is no one study alone that meets impeccable standards for exposure assessment or totally eliminates all possibility for bias, but the constellation of studies together gives adequate support to delineate a ‘lowest observed effects level’, that in turn, with added safety margins, can serve as a guideline for precautionary action.

A reduction from the BioInitiative 2007 recommendation of 0.1 uW/cm2 (or one-tenth of a microwatt per square centimeter which is the same as 100 nanowatts/cm2) for cumulative outdoor RFR down to something three orders of magnitude lower (in the low nanowatt per square centimeter range) is justified on a public health basis. We use the new scientific evidence documented in this Report to identify ‘effect levels’ and then apply one or more reduction factors to provide a safety margin. We do note however, even a precautionary action level of several tenths of a nanowatt per square centimeter (or several hundred picowatts per square centimeter) would still allow for cell phone transmissions (that can operate down to about 0.00003 V/m).

Even so, these levels may need to go lower in the future, as new and better studies are completed. This is what the authors said in 2007 (Carpenter and Sage, 2007, BioInitiative Report) and it remains true today in 2012. We leave room for future studies that may lower today's observed 'effects levels' and should be prepared to accept new information as a guide for new precautionary actions.

Establishing A Scientific Benchmark for 'Lowest Observed Effect Levels'

Studies that provide information at 'new levels of observed effect' have been identified. These serve as scientific benchmarks for possible risk to health and well-being. Next, we indentify reduction factors to compensate for sensitive subpopulations and apply them to the scientific benchmarks (lowest observed effect levels).

A ten-fold reduction factor is warranted (or higher) for studies that report effects from only short-term (i.e., acute) rather than chronic (i.e., long-term) exposures. Longer duration of exposure can cause bioeffects at lower exposures where these effects are NOT seen with shorter (acute) exposures (Belyaev, 1997; Belyaev, 2012). Chronic exposures with longer durations of weeks, months or years is what most populations face with respect to wireless classrooms, wireless offices and locations near base stations.

A second ten-fold reduction (or higher) is justified as a buffer for sensitive populations including children, the elderly and other adult groups that may be ill, already sensitized, in remission or suffer from ailments made worse by physiological stress and insomnia.

Studies which contribute together can reasonably contribute to delineating a new RFR lower effects level are primarily mobile phone (cell phone) base station studies of healthy human populations and studies of sperm damage in men who use and/or wear their wireless devices on or around the belt or pants pocket.

Power Density Studies (Mobile Phone Base Stations and Sperm/Fertility Studies)

A scientific benchmark of 0.003 uW/cm2 or three nanowatts per centimeter squared for ‘lowest observed effect level’ for RFR is based on mobile phone base station-level studies. The Thomas et al, (2008) study shows effects at a LOEL of 0.005 uW/cm2 on adults exposed to short-term cell phone radiation only (it is not a chronic exposure study). Other studies that are relevant are Thomas et al (2010) with a LOEL of 0.003 uW/cm2 and Heinrich et al (2010) with a LOEL of 0.003 uW/cm2. Both studied mixed child/adolescent populations of students, but have short-term test periods (are not chronic exposure studies) and have LOELs of 0.003 uW/cm2. Buchner et al (2012) shows a 0.006 uW/cm2 ‘effect level’ and tests adult populations, but achieves ‘chronic’ exposure testing criterion (over 18 months). Applying a ten-fold reduction to compensate for the lack of long-term exposure (to provide a safety buffer for chronic exposure) or for children as a sensitive subpopulation yields a 300 to 600 picowatts per square centimeter precautionary action level. This is also equal to a 0.3 nanowatts to 0.6 nanowatts per square centimeter as a reasonable, precautionary action level.

Of the studies that deal with children and base-station level RFR exposures, none studied children exclusively, so the results may dilute out any apparent effects accruing to the younger test subjects. Thomas et al (2010) is a short-term exposure study of children and adolescents 8 to 17 years in age. Heinrich et al (2010) is a further study of the same population of 8 to 17 year olds over the short-term. A 100-fold reduction could be defended as reasonably conservative in this instance.

Behari et al (2006) provides the one sperm study expressed in power density units with a LOEL of 0.00034 uW/cm2. It is a chronic exposure study. The majority of sperm studies with good exposure information are expressed in SARs (W/kg). These range from LOELs of 0.014 (Kumar et al, 2012) to 0.091 W/kg (Atasoy et al, 2012) to 0.43 W/kg (Salama et al, 2008) to 0.795 W/kg (Panagopoulous et al, 2012) to 0.9 W/kg (Kesari et al, 2012). All the other sperm damage or ovarian damage studies have SARs of greater than 1.0 W/kg (7 more studies). All are short-term studies. There are more sperm damage studies but without any measurements or other specific exposure information. These are studies that place sperm, or mice, or give prenatal exposures to animals close to sources of cell phone radiation. Such studies give weight to the argument that low-intensity RFR exposures can cause damage, but do not help in delineating LOELs because they have no specific exposure numbers, just distances.

Most of the sperm studies and base station studies which have exposures expressed power density (microwatts per square centimeter) have 'effect' levels in the nanowatt range (0.34 nanowatt/cm2 to 100 nanowatt/cm2)*. They include Behari and Kesari, 2006; Buchner and Eger, 2012; Oberfeld et al, 2004; Thomas et al, 2008, 2010; Heinrich et al, 2010; Navarro et al, 2003; and Otitoloju et al 2010. Avendano et al (2012) report that WI-FI exposure from a 4-hr laptop exposure decreased sperm viability and caused DNA fragmentation in human sperm samples (exposure in petri dishes) at 0.5 to 1.0 uW/cm2. The Kundi-Hutter 2009 Pathophysiology Journal review paper of base station studies through 2006 reports an overall NOEL below 0.05 to 0.1 uW/cm2. Overall, the new 2007-2012 power density studies are reporting ‘lowest effects levels’ two or three orders of magnitude lower than in 2006, down from the microwatt/cm2 range to the nanowatt/cm2 range.

SAR Studies (Sperm Studies and Ovarian Damage with Cell Phone Radiation Exposures)

Studies on male fertility (adverse effects on sperm, on the testes size and morphology, etc) coming from cell phone-in-the-pocket-on-stand-by-mode and wireless laptop studies provide us with a flood of new data showing very low-intensity effects to guide precautionary actions and to educate the public about potential risks to health, fertility and reproduction.

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*The RF Color Charts in this Report are a guide to reported biological effects and those RFR levels reported to cause them.

Sperm and fertility studies with ‘effects levels’ in the 9 microwatt/kg to 80 milliwatt/kg range are Kumar et al (2012) (male infertility) and Aitken et al (2005) (sperm DNA damage). Sperm studies with ‘effect levels’ in the 90 to 900 milliwatt/kg range are De Iuliis et al (2009) (human sperm cell damage), Salama et al (2008) (decrease in sperm mobility and concentration), Panagopoulous et al (2012) (ovarian damage) and Kesari et al (2012) (sperm damage). Studies from 1 W/kg to 1.8 W/kg that report sperm or reproductive damage are Gul et al (2009) (toxic effect on ovaries), Agarwal et al (2008) (sperm damage), Agarwal et al (2009) (sperm damage) and Yan et al (2007) (deformed sperm cells, disabled for swimming).

The WI-FI laptop study by Atasoy et al (2012) reports that exposures to laptops estimated at 0.091 W/kg increase DNA damage and reduce DNA repair in damaged sperm, and “*raise questions about safety of radiofrequency exposure from WI-FI internet access dvices for growing organisms of reproductive age, with a potential effect on fertility and integrity of germ lines*.”

Altered fetal development in mice exposed to RFR at SARs of 0.3 to 60 milliwatt/kg is reported to result in consequent adverse effects on learning and behavior (Aldad et al, 2012). Fragopoulou et al (2009) reported changes at 600 to 900 milliwatts/kg in mouse embryos.

General Approach to Delineating a Precautionary Action Level

As a methodology, is not necessary or wise to use an averaging approach among studies. The technique itself is too vulnerable to weighting problems by the older studies that did not test for effects at the lowest range of exposures to RFR (or did not have the power to assess effects). Averaging also is insensitive in giving proper visibility to important NEW results at the very low-intensity (nanowatt, picowatt and femtowatt/cm2 range). Even when they are averaged together, these studies contribute vanishingly small influence when averaged together with studies of much higher power density to determine a scientific benchmark for harm.

One limitation of the sperm studies using base station-level RFR exposures is that good estimates of exposure are available if sperm are tested outside the body (in petri dishes), but that does not reflect the more realistic situation of sperm exposed in humans themselves (using or carrying a mobile phone near the testes) where exposure estimates are more difficult to determine. So, it is useful and informative to observe the combined results of both in-vivo and ex-vivo studies as a guide. For base station studies on human populations, the quality of exposure assessments is variable, and in some cases inadequate. Further, very few base station studies are conducted so that test subjects do not know if/when they are subjected to elevated RFR (blinded studies), so that some bias may influence results. People often report more ill effects because they are aware of the exposure (from a nearby base station, for example). These variations in quality across the studies, however, do not offset their usefulness in the aggregate for delineating what the lowest observable effect exposures are, and helping to guide decision-making for public health and precautionary actions.

A further concern is that time-averaging of RFR to give a single numeric recommendation for a precautionary action guideline does not address the critical difference between peak power levels (RFR spikes that occur intermittently) and measurements that hide how high peak power spikes are by dilution. Biological responses can last over seconds of time, or have even longer effects on proteins and enzymes, while the RFR pulses may be in microseconds or milliseconds in duration. It is entirely possible that what causes bioeffects is the high, intermittent RFR spikes that the body perceives and responds to as one continuous, high-power assault. For example, the DECT phone peak power is about 100 times larger than what RFR is measured with time-averaging. A person near a cell tower that produces an RFR measurement of 0.1 microwatts/cm2 is probably getting RFR power density spikes of eight times higher, if one could measure the spikes individually. None of the studies profiled in this section deal with peak power pulses and biological response times that are longer than the ‘intermission’ between RFR spikes. Thus, precautionary action levels should err on the side of being conservative.

The planning of base stations, and other site evaluations needs to have a scientific benchmark below which effects have not (not yet) been characterized, published or vetted. Then, a reasonable safety buffer should be added - remembering that the design life of such facilities may be 30-50 years long. This is standard procedure for environmental planning constraints.

Health Agencies Should Act Now

Health agencies and regulatory agencies that set public safety standards for ELF-EMF and RFR should act now to adopt new, biologically-relevant safety limits that key to the lowest scientific benchmarks for harm coming from the recent studies, plus a lower safety margin. Existing public safety limits are too high by several orders of magnitude, if prevention of bioeffects and resulting adverse health effects are to be minimized or eliminated. Most safety standards are a thousand times or more too high for healthy populations, and even less effective in protecting sensitive subpopulations.

New, biologically-based public exposure standards are critically needed now and should key to scientific benchmarks for harm, plus a safety margin below that level.

Standard of Evidence for Judging the Science

The standard of evidence for judging the scientific evidence should be based on good public health principles rather than demanding scientific certainty before actions are taken.

Sensitive Populations Require Special Protections

Safety standards for sensitive populations will need to be set at lower levels than for healthy adult populations to protect the developing fetus, the infant and young child, school-age children, the elderly, those with pre-existing chronic diseases, and those with developed electrical sensitivity (EHS). Men of child-bearing age should not wear wireless devices on their body in order to protect the integrity of sperm DNA. Sperm should be considered a ‘sensitive population’. Scientific benchmarks for lowest effect levels should be identified, and applied with additional safety margin reductions to safeguard populations against excessively high exposure to chronic ELF-EMF and RFR.

Protect Children Against Chronic Exposure to Wireless Devices

Strong precautionary action and clear public health warnings are universally warranted for use of cordless and cell phones to help prevent a global epidemic of brain tumors. This is especially important for children, adolescents and young adults, while new safety standards are established and implemented. Children should not use wireless devices except in the case of emergencies, or be exposed on an involuntary and chronic basis to wireless in their living, sleeping or learning environments.

Common Sense Precautionary Measurees are Warranted Now

Common sense measures to limit both ELF-EMF and RFR in the fetus and newborn infant are needed, especially with respect to avoidable exposures like baby monitors in the crib and baby isolettes (incubators) in hospitals that can be modified; and where education of the pregnant mother with respect to laptop computers, mobile phones and other sources of ELF-EMF and RFR are easily instituted.

Wireless laptops and other wireless devices should be strongly discouraged in schools for children of all ages, and wireless systems already installed should be replaced with wired (cable) alternatives. While without question it is important for children to have access to the internet, wired computer laboratories will have no elevated exposure to RFR. What might be lost in flexibility of moving rooms arounds will be more than gained by reducing exposure to RFR if wired connections, rather than wireless, are used. Pregnant women should be strongly cautioned not to use wireless devices during pregnancy. If a school already has wireless facilities, classrooms without wireless should be made available to students, teachers and staff during the transition if sensitivities to EMF are reported by the individual. Special education classroom teaching environments should offer wired teaching environments (not wireless), nor should they be exposed to off-site wireless radiofrequency radiation from other sources that elevate interior levels for children.

Special Protections for the Integrity of the Genome and Reproduction

Reducing life-long health risks should begin in the earliest stages of embryonic and fetal development. Development pace is accelerated for the infant and very young child compared to adults, and is not complete in young people (as far as brain and nervous system maturation) until the early 20's. Windows of critical development mean that risk factors once laid down in the cells, or in epigenetic changes in the genome may have grave and life-long consequences for health or illness for every individual, and furthermore these genetic and epigenetic changes may be passed to the next generation. All relevant environmental conditions, including biologically active exposures to EMF and RFR that can degrade the human genome, and impair normal health and development of all species including humans - should be given weight in defining and implementing strong precautionary actions now to protect public health. The consequence of ignoring clear evidence of large-scale health risks to global populations, when the risk factors are largely avoidable or preventable is too high a risk to take.

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SECTION 25

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SECTION 26

Glossary of Terms and Abbreviations

Prepared for the BioInitiative Working Group

July 2007

Absorption. In radio wave propagation, attenuation of a radio wave due to dissipation of its energy, i.e., conversion of its energy into another form, such as heat.

Athermal effect. Any effect of electromagnetic energy on a body that is not a heat-related effect.

Blood–brain barrier. A functional concept developed to explain why many substances that are transported by blood readily enter other tissues but do not enter the brain; the "barrier" functions as if it were a continuous membrane lining the vasculature of the brain. These brain capillary endothelial cells form a nearly continuous barrier to entry of substances into the brain from the vasculature.

Conductance. The reciprocal of resistance. Expressed in siemens (S).

Conductivity: A property of materials that determines the magnitude of the electric current density when an electric field is impressed on the material.

Continuous wave. A wave whose successive oscillations are identical under steady-state conditions.

Current density. A vector of which the integral over a given surface is equal to the current flowing through the surface; the mean density in a linear conductor is equal to the current divided by the cross-sectional area of the conductor. Expressed in ampere per square metre (A m^{-2}).

Depth of penetration. For a plane wave electromagnetic field (EMF), incident on the boundary of a good conductor, depth of penetration of the wave is the depth at which the field strength of the wave has been reduced to 1/e, or to approximately 37% of its original value.

Dielectric properties: In the context of this document the properties of materials conductivity and permeability.

Dosimetry. Measurement, or determination by calculation, of internal electric field strength or induced current density, of the specific energy absorption, or specific energy absorption rate distribution, in humans or animals exposed to electromagnetic fields.

Electric field strength. The force (**E**) on a stationary unit positive charge at a point in an electric field; measured in volt per metre (V m^{-1}).

Electrosensitivity (Electrohypersensitivity): A working definition of EHS from Bergqvist et al. (1997) is "a phenomenon where individuals experience adverse health effects while using or being in the vicinity of devices emanating electric, magnetic or electromagnetic fields (EMFs)".

Electromagnetic energy. The energy stored in an electromagnetic field. Expressed in joule (J).

Electric field strength (E): The magnitude of a field vector at a point that represents the force (F) on a charge (q). E is defined as E = F/q and is expressed in units of Volt per meter (V/m).

Electromagnetic field: Electromagnetic phenomena expressed in vector functions of space and time.

Electromagnetic radiation: The propagation of energy in the form of electromagnetic waves through space.

EMF. Electric, magnetic, and electromagnetic fields.

Exposure: Exposure occurs wherever a person is subjected to electric, magnetic or electromagnetic fields or contact currents other than those originating from physiological processes in the body.

Extra low frequency (ELF): Extra low frequency fields include, in this document, electromagnetic fields from 1 to 300 Hz. Alternately, **ELF-** Extremely low frequency where the European convention is extremely low frequncy, US is extra-low frequency.

Frequency modulation (FM): Frequency Modulation is a type of modulation representing information as variations in the frequency of a carrier wave. FM is often used at VHF frequencies (30 to 300 MHz) for broadcasting music and speech.

Far field. The region where the distance from a radiating antenna exceeds the wavelength of the radiated EMF; in the far-field, field components (**E** and **H**) and the direction of propagation are mutually perpendicular, and the shape of the field pattern is independent of the distance from the source at which it is taken.

Frequency. The number of sinusoidal cycles completed by electromagnetic waves in 1 second; usually expressed in hertz (Hz).

Impedance, wave. The ratio of the complex number (vector) representing the transverse electric field at a point to that representing the transverse magnetic field at that point. Expressed in ohm (S).

Magnetic flux density (B): The magnitude of a field vector at a point that results in a force (F) on a charge (q) moving with the velocity (v). The force F is defined by F = q*(v x B) and is expressed in units of Tesla (T).

Magnetic field strength (H): The magnitude of a field vector that is equal to the magnetic flux density (B) divided by the permeability (μ) of the medium. H is defined as H = B/μ and is expressed in units of Ampere per metre (A/m).

Magnetic permeability. The scalar or vector quantity which, when multiplied by the magnetic field strength, yields magnetic flux density; expressed in henry per metre (H m$^{!1}$*).* *Note:* For isotropic media, magnetic permeability is a scalar; for anisotropic media, it is a tensor quantity.

Microwaves: Microwaves are defined in the frame of this expertise as electromagnetic waves with wavelengths of approximately 30 cm (1 GHz) to 1 mm (300 GHz).

Milligauss (mG): A milligauss is a measure of ELF intensity and is abbreviated mG. This is used to describe electromagnetic fields from appliances, power lines, interior electrical wiring.

Milliwatt (mW): A unit of power equal to 10^{-3}.

Microwatt (uW): A unit of power equal to 10^{-6}

Microwatts per centimeter squared (µW/cm2)

Radiofrequency radiation in terms of power density is measured in microwatts per centimeter squared and abbreviated (µW/cm2). It is used when talking about emissions from wireless facilities, and when describing ambient RF in the environment. The amount of allowable RF near a cell tower is 1000 µW/cm2 for some cell phone frequencies, for example.

Nanowatt (nW): A unit of power equal to 10^{-9} Watt.

Non – thermal effects (or athermal effects): An effect which can only be explained in terms of mechanisms other than increased molecular motion (i.e. heating), or occurs at absorbed power levels so low, that a thermal mechanism seems unlikely, or displays so unexpected a dependence upon some experimental variable that it is difficult to see how heating could be the cause.

Near field. The region where the distance from a radiating antenna is less than the wavelength of the radiated EMF. *Note:* The magnetic field strength (multiplied by the impedance of space) and the electric field strength are unequal and, at distances less than one-tenth of a wavelength from an antenna, vary inversely as the square or cube of the distance if the antenna is small compared with this distance. Near field exposures are unreliable for estimation of exposures by calculation. The can zero out or be additive and nearly infinite, thus creating problems for exposure assessment.

Non-ionizing electromagnetic radiation (NIER). Includes all radiations and fields of the electromagnetic spectrum that do not normally have sufficient energy to produce ionization in matter; characterized by energy per photon less than about 12 eV, wavelengths greater than 100 nm, and frequencies lower than 3×10^{15} Hz.

Occupational exposure. All exposure to EMF experienced by individuals in the course of performing their work. Safety limits are five times higher for allowable occupational exposures than for general public exposures in the US.

Permeability (µ): A property of materials that indicates how much polarisation occurs when an electric field is applied.

Permittivity. A constant defining the influence of an isotropic medium on the forces of attraction or repulsion between electrified bodies, and expressed in farad per metre (F $m^{!1}$); *relative permittivity* is the permittivity of a material or medium divided by the permittivity of vacuum.

Public Exposure. All exposure to EMF experienced by the general public excluding exposure during medical procedures and occupational work environments. Public exposure limits in the US are five times lower than for occupational exposures, where informed consent by employees is required.

Power Density. The power as measured in free space (ambient) as opposed to measured by SAR or specific absorption rate (within tissues or the body). The unit of measurement can be watts per square meter, milliwatts per square meter or microwatts per centimeter squared. Radiofrequency (RF). Any frequency at which electromagnetic radiation is useful for telecommunications, or broadcasting for radio and television. Frequency range is usually defined as 300 Hz (300 hertz) to 300 GHz (300 gigahertz).

Radiofrequency (RF): The frequencies between 100 kHz and 300 GHz of the electromagnetic spectrum.

Reasonance. The change in amplitude occurring as the frequency of the wave approaches or coincides with a natural frequency of the medium; whole body absorption of electromagnetic waves presents its highest value, i.e., the reasonance. for frequencies (in MHz or megahertz) corresponding to approximately 114/L where L is the height of the individual in meters. Reasonance can also be applicable to organs, tissues, or other body parts.

Specific Absorption Rate (SAR is measured in watts per kilogram or W/Kg)
SAR stands for specific absorption rate. It is a calculation of how much RF energy is absorbed into the body, for example when a cell phone or cordless phone is pressed to the head. SAR is expressed in watts per kilogram of tissue (W/Kg). The amount of allowable energy into 1 gram of brain tissue from a cell phone is 1.6 W/Kg in the US. For whole body exposure, the exposure is 0.8 W/Kg averaged over 30 minutes for the general public. International standards in most countries are similar, but not exactly the same.

Static electric field: Static fields produced by fixed potential differences.

Static magnetic fields: Static fields established by permanent magnets and by steady currents.

VDU: Video display units for computers, videos, TV and some measurement devices using cathode ray tubes

WI-FI: Stands for wireless fidelity. WI-FI systems create zones of wireless RF that allow access to wireless internet for computers, internet phone access and other wireless services. Access points that provide WI-FI to access Land Area Networks (LANs) can be installed on streets (for city-wide coverage) or indoors in buildings, Restaurants, hotels, coffee shops, airports, malls and other commercial enterprises are widely installing WI-FI. The range of typical WI-FI systems is about 300 feet.

WI-MAX: Stands for "Wireless interoperability for Microwave Access" and is a telecommunications technology aimed at providing wireless data over long distances. Like WI-FI, WI-MAX systems are designed to provide wireless access but over much broader geographic areas, with some systems transmitting signal up to 10 miles. Higher levels of RF are produced at the wireless transmission facilities than for WI-FI.s

Section 20 LIST OF ABBREVIATIONS

µT	microtesla
µW	microwatt
AC	Alternating current
ALS	Amyotrophic Lateral Sclerosis
AM	Amplitude modulation
B	Magnetic flux density
BBB	Blood-Brain-Barrier
CENELEC	European Committee for Electrotechnical Standardization
CI	Confidence Interval
CNS	Central Nervous System
CW	Continuous wave
DC	Direct current
DECT	Digital Enhanced Cordless Telephone
DMBA	7,12-dimethylbenz[a]anthracene
DNA	Deoxyribonucleic acid
EEG	Electroencephalogram
EHS	Electromagnetic hypersensitivity
ELF	Extra low frequency (also ELF-EMF)
EMF	Electromagnetic field
FM	Frequency Modulation
GSM	Global System for Mobile Communication
H	Magnetic field strength
HSP	Heat-shock proteins (stress proteins)
Hz	Frequency in Hertz
IARC	International Agency for Research on Cancer
IL	Interleukin
kg	Kilogram
kHz	Kilohertz
kV	Kilovolt
MF	Magnetic Field (sometimes MF-ELF)
MHz	Megahertz
ms	Milliseconds
mT	Millitesla
mG	Milligauss
mW	Milliwatt
nT	Nanotesla

nW	Nanowatt
NRPB	National Radiation Protection Board (HPA)
OR	Odds Ratio (measure of increased risk of disease)
REFLEX	European Research Program for Radiofrequency Hazards
RF	Radiofrequency Radiation (also written as RFR or RF-EMF)
SCENIHR	Scientific Committee on Emerging and Newly Identified Health Risks
TNO	Nederlandse Onderzoek (Netherlands Organisation Applied Scientific Research
UMTS	Universal Mobile Telephony System **UNEP** United Nations Environmental
VDT	Video display terminal (VDU – for computers, videos, TV, that use cathode ray tubes).
Wi-Fi	Short for wireless fidelity – wireless internet access - works for short- distances for cell phone and laptop computer access without wires.
WLAN	Wireless Local Area Network (wireless internet coverage usually up to 300' provided by access points that create elevated radiofrequency radiation for that service zone.
WiMAX	Worldwide Interoperability for Microwave Access (wireless service up to 10 miles in comparison to Wi-Fi that may serve 300' area)
WHO	World Health Organisation
FCC	The Federal Communications Commission (FCC) is an independent United States government agency, created, directed, and empowered by Congressional statute to oversee the regulation of radio and TV broadcasting and wireless technologies. It is not a health agency.
HPA	Health Protection Agency (UK) that was formerly the National Radiation Protection Division Board). The Health Protection Agency (HPA) is an independent body that protects the health and well-being of the population. The Agency plays a critical role in protecting people from infectious diseases and in preventing harm when hazards involving chemicals, poisons or radiation occur.
DNA	Deoxyribonucleic acid, or DNA is a nucleic acid molecule that contains the genetic instructions used in the development and functioning of all living things.
Melatonin	Melatonin is a hormone produced in the brain by the pineal gland, It is a potent anti-oxidant that protects against oxidative damage from free radicals that can cause DNA damage.
Alzheimer's	Alzheimer's disease is a progressive brain disorder that gradually destroys a person's memory and ability to learn, reason, make judgments, communicate and carry out daily activities. As Alzheimer's progresses, individuals may also experience changes in personality and behavior, such as anxiety, suspiciousness or agitation, as well as delusions or hallucinations.

RFIAWG	Radiofrequency Interagency Working Group (US) composed of members from federal agencies with some interest in radiofrequency radiation issues. This Working Group was made up of representatives from the US government's National Institute for Occupational Safety and Health (NIOSH), the Federal Communications Commission (FCC), Occupational Health and Safety Administration (OSHA), the Environmental Protection Agency (US EPA), the National Telecommunication and Information Administration, and the US Food and Drug Administration (FDA).
ICNIRP	International Commission on Non-Ionizing Radiation. It is a body of independent scientific experts consisting of a main Commission of 14 members, 4 Scientific Standing Committees covering Epidemiology, Biology, Dosimetry and Optical Radiation and a number of consulting experts. This expertise is brought to bear on addressing the important issues of possible adverse effects on human health of exposure to non-ionising radiation.

SECTION 27

Appendix 20-A Average Residential Exposures to ELF (Power Frequency Fields)

Prepared for the BioInitiative Working Group

July 2007

What are Ambient ELF and RF Levels?

A nation-wide survey in the United States by Zaffanella et al (1993) collected engineering data on sources and levels of 60 Hz electric power magnetic fields that exist inside residences in the United States.

Approximately 1000 residences were randomly selected for the survey. The goals were to 1) identify all significant sources of magnetic field, 2) estimate for each source the percentage of residences where magnetic fields exceeded specified levels, 3) to determine the relation between magnetic field and sources and 4) to characterize the field varations in time.

The median field was identified as 0.5 mG and the average field was 0.9 mG. Thus, this confirms that average residential magnetic fields based on the 1000-home study is less than 1 mG.

Appliances produce magnetic fields but these diminish rapidly with distance (at $1/R^3$),

Power lines generally produce the largest average residential magnetic field when the entire living space of a residence and a 24-hour period are considered. Power line magnetic field exceeds 1 mG in 17%, exceed 2.5 mG in 9.5% and exceed 5 mG in 0.3% of all the residences surveyed.

Zaffanella (1998) conducted measurements to characterize typical EMF exposure levels in persons living in the United States - a study called the 1000-Person Study. Table A-S.2 shows that about half of all people in the US have EMF exposures at home under 0.75 mG; in bed are 0.48 mg; at school 0.60 mG; at work 0.99 mG; and 0.87 mG is the median EMF exposure for an average 24-hour day.

Table A-S.2

Table S.2 Descriptive Statistics for Different Activity Periods

Parameter	Home not in Bed	In Bed	Work	School	Travel	24-Hour
Number of Valid Data Sets	1011	996	525	139	765	1012
1st Percentile	0.10 mG	0.01 mG	0.14 mG	0.13 mG	0.13 mG	0.18 mG
5th Percentile	0.20 mG	0.08 mG	0.24 mG	0.18 mG	0.29 mG	0.27 mG
10th Percentile	0.27 mG	0.12 mG	0.30 mG	0.29 mG	0.41 mG	0.35 mG
25th Percentile	0.44 mG	0.24 mG	0.60 mG	0.35 mG	0.66 mG	0.51 mG
50th Percentile	0.75 mG	0.48 mG	0.99 mG	0.60 mG	0.98 mG	0.87 mG
75th Percentile	1.39 mG	1.24 mG	1.78 mG	1.01 mG	1.46 mG	1.41 mG
90th Percentile	2.49 mG	2.44 mG	3.32 mG	1.64 mG	2.18 mG	2.38 mG
95th Percentile	3.89 mG	3.63 mG	5.00 mG	1.77 mG	2.73 mG	3.38 mG
99th Percentile	9.50 mG	9.19 mG	13.5 mG	3.55 mG	5.43 mG	6.16 mG
Mean	1.29 mG	1.11 mG	1.73 mG	0.82 mG	1.22 mG	1.25 mG
Standard Deviation	2.54 mG	2.06 mG	3.09 mG	0.70 mG	0.99 mG	1.51 mG
Geometric Mean	0.80 mG	0.52 mG	1.03 mG	0.64 mG	0.96 mG	0.89 mG
Geometric Standard Deviation	2.50	3.52	2.57	2.06	2.03	2.18

In Sweden, Mild et al (1996) report that overall mean residential ELF exposures are 0.4 mG, and in Norway are 0.13 mG.

Average Occupational Exposures to ELF

Average occupational exposures in commercial office buildings are 1-2 mG or less and have been reported fairly consistently across numerous studies of exposure assessment (Table 1). Powerline and electrical workers have higher average occupational exposures from 10 mG to 16.6 mG.

Table A-2: Average Occupational Exposures to ELF

EMF RAPID Program – Questions and Answers, NIEHS, June 2002	
Office buildings (median)	0.6 mG
Support staff	0.5 mG
Professional staff	0.6 mG
Maintenance staff	0.6 mG
Visitors	0.6 mG
EMF RAPID Program Engineering Project #3 Executive Summary, May 1996	
Office building (average)	0.7 mG
Office building (median)	0.4 mG
Electric and Magnetic Field Fundamentals (EPRI Resource Paper, March 1994)	
Typical magnetic fields in offices	1 – 2 mG
Power line workers	10 mG
Occupational EMF Exposure Assessment (EPRI Resource Paper, February 1994)	
Office Worker Comparison Group	1.6 mG
All Occupationally Exposed Utility Workers	16.6 mG
Table 7 – Other Studies Cited	
Bracken Study (1990)	1.0 mG
Deadman Study (1988)	1.6 mG
Bowman Study (1992)	0.9 – 1.8 mG

Limits on Operation of Sensitive Electronic Equipment

Companies that manufacture or use equipment in nanotechnology and biotechnology and found 1.0 mG is generally the limit for proper operation of electron beam devices (mass spectrometers, scanning electron microscopes, lithography, etc) used in these technologies. Ten (10) milligauss (mG) is the EMF limit for normal computers – above 10 mG can introduce “computer jitter” and other problems.

What are Ambient Radiofrequency Radiation/Microwave Levels?

Prior to the rapid development of wireless communications for personal and business usage, RF power density levels were primarily related to AM, FM and television broadcasting signal in both urban and rural areas of the United States. Microwave frequencies used for wireless communications were negligible.

Original extra-planetary sources of microwave radiation were infinitesimally small, on the order of a billionth of a microwatt per centimeter squared (10^{-12} uW/cm^2). Human evolution took place without any appreciable exposure to microwave radiation from background sources. The human body has no evolutionary protection against microwave radiation, as it does for ultraviolet radiation from the sun (Johannson, 2000). Wireless voice and communications have introduced unprecedented levels of public exposure in the last decade.

Mantiply (1997) measured and reported common sources and levels of RF in the environment. He identified areas near cellular base stations on the ground near towers to be from 0.003 to 0.3 µW/cm2. Background level ambient RF exposures in cities and suburbs in the 1990's were generally reported to be below 0.003 µW/cm2.

Hamnerius (2000) reported that ambient RF power density measurements in twelve (12) large cities in Sweden were roughly ten times higher than in the United States for equivalent measurement locations by Mantiply in 1978 (when no cellular phone service existed in the US). He reported a total mean value of 26 measured sites in the study was 0.05 µW/cm2 and the median value was 40 µW/cm2. An office location with a base station nearby at about 300 feet distance tested 150 µW/cm2. A train station with antennas mounted indoors tested at about 3 µW/cm2. Both indoor and outdoor ambient RF power density measurements showed high variability depending on proximity to transmitting antennas.

Sage Associates reported on microwave frequency RF power density levels at outdoor locations both near and far from wireless antenna sites in the United States (Sage, 2000). Within the first 100-300 feet, power density levels have been measured at 0.01 to 3.0 µW/cm2. Elevated RF power density levels from a major wireless antenna site can often be detected at 1000 feet or more. Power density levels away from wireless antenna sites measure between 0.001 µW/cm2 to 0.000001 µW/cm2. Vegetation often reduces signal (and therefore the reach of elevated RF exposures) but dry building materials used to visually screen wireless sites do not appreciably diminish signal transmission. Therefore, many sites that are "out-of-sight" because of stealth design can still produce elevated RF levels in nearby areas where people live, work and go to school. For purposes of this evaluation, a 10 dB attenuation has been incorporated to take building material shielding effects into account.

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APPENDIX 20-B

STANDARDS OF EVIDENCE FOR DECISIONMAKING DIFFERS AMONG PROFESSIONS

There is a large difference between what constitutes causal evidence for purposes of achieving scientific consensus, what constitutes sufficient evidence for purposes of interim public health policy, and what constitutes "a more likely than not" case. A central confusion in this debate is whether prudent policy and public health decisions necessarily require conclusive scientific evidence first. This is not the case. The state of the science needs to be presented in an understandable and scientifically accurate manner, but prudent public health actions do not and should not require 100% proof of harm. In fact, precautionary and preventative actions are specifically justified at a point in time before scientific proof is established. If the growing weight of evidence is positive (although all studies need not report positive effects) then it may be essential to take preventative actions and implement policies that are protective of public health, safety and welfare rather than wait for absolute certainty. The following discussion is presented to highlight some of the main differences in professional approach and traditional ways of viewing and interpreting scientific evidence. In reality, the basis for taking action (preventative or precautionary action) is a continuum – there are no hard and fast rules. The bar for Public Health Policy may be higher or lower than shown in Figure 2; based on many factors, including how widespread the risk, how dread the disease, the cost of inaction (doing nothing until there is proof, but many may be harmed), etc.

A. Scientific Standard of Evidence

There are several levels of proof for adverse effects of environmental exposures. The most rigorous is a scientific standard, where virtual proof of causation is typically required by scientists to arrive at consensus about an effect. This approach works best in physics and chemistry. In biological systems this is rarely possible.

In this case, the 'scientific standard' refers to the overall evidence that the science community typically requires before rendering opinions on the strength of evidence, and what evidence they believe is necessary to establish a causal link (proof).

Figure 1 shows Standards of Evidence that are routinely employed by various interest groups in the EMF debate (Sage, 1997). It can be used to focus on various accepted standards for evidence that are legitimately used by scientific and professional groups to determine when an action is appropriate. The varying levels of certainty about an outcome will dictate different decision-making among different groups that may all be appropriate given their professional charge. Even though the evidence required to make a scientific determination about causality has a far higher standard than a legal determination on the 'weight of the evidence' or 'preponderance of evidence' (a legal standard), neither negates the correctness of the other in its proper jurisdiction. Scientists typically want all possible evidence (animal, cell and epidemiological studies, with replications) showing a high degree of consistency. This can generally be described as a 95% to

99% degree of certainty before drawing conclusions (it does not refer to the 95% confidence interval in epidemiology, except as a part of the overall proof).

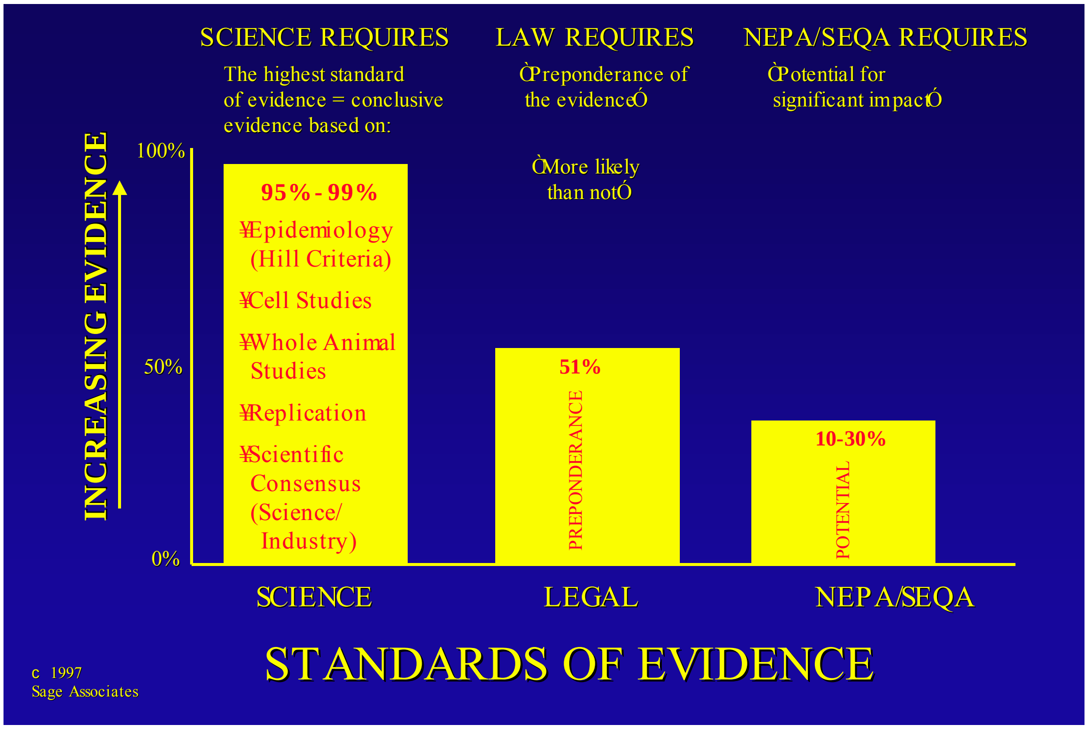

Figure 1 Variable Standards of Evidence (By Profession)

B. Legal Standard of Evidence

The second level of proof is the standard applied in legal proceedings, which is ‘more likely than not’ or ‘preponderance of the evidence’ (Figure 1). This is to say if there is a 50%+ likelihood of harm, this is taken as evidence for a relationship (Sage, 1997). At least this level of evidence is reached for the studies of adult cancer and neurodegenerative diseases and 50/60 Hz magnetic field exposures. As with childhood leukemia, while we have documented associations, this does not necessarily indicate causation. Failure to meet either the scientific or the legal standard of proof does not mean that there is no relationship between exposure and disease. In the case of EMF exposure, where everyone is exposed, the societal implications may be huge if there is a real risk whose magnitude has simply just not yet been clarified. Public policies are needed to address this issue of decision-making in the face of this scientific uncertainty.

C. Environmental Protection Standard of Evidence

National and state environmental quality acts (The National Environmental Policy Act) and various state environmental quality acts (SEQA) require that assessments use a standard of "potential for a significant impact on the environment which is a relatively low level of certainty (10% to 30%). The potential for a significant impact requires that mitigation strategies be developed, i.e, require precautionary or preventative actions when only the potential for risk is present (Figure 1).

For example, the potential for risk to humans from building on an active earthquake fault will require a finding of potentially significant impact, and will require mitigative action; even when there is no certainty (no causal evidence) that the fault will rupture and cause damage within the design lifetime of the building. Proof of harm is not a pre-condition for taking action, and the level of certainty is low in comparison to a scientific or legal standard of certainty. Nonetheless, each standard has validity, and will have a different level of evidence required to take action. What decision-makers need to address is what standard of evidence is appropriate now to guide them with respect to EMF exposures that are clearly of environmental and public health concern.

D. Public Health Standard of Evidence

The prudent approach from a public health point of view is to take preventive actions as if causation had been proven, while at the same time to continue to search for mechanisms of action. In the case of childhood leukemia and ELF exposure there is a consistent and statistically significant association in most studies, while for many of the other diseases the results are less consistent although strong associations are found in some studies (Figure 2). This bar graph should be considered illustrative only, since the level of certainty may be higher or lower (above or below 50%) depending on the circumstances of the potential risk, and costs of those risks to society.

Whether magnetic fields actually cause childhood leukemia and the other cancers and neurological diseases documented in this Report; or whether it is some other component in the electromagnetic environment that is responsible for the association is a subject of debate within the scientific community, but from a public health point of view it doesn't matter. The fact that there are unknowns does not negate or override the ultimate public health responsibility, which is to protect the population from exposures which cause disease. Those who make public health decisions, as well as policymakers who rely on them and who approve construction of new schools and homes near power lines, those who provide insurance or financing of new construction, those who must choose siting routes for new electrical facilities all face making decisions with some uncertainty about the potential health risks from EMF exposure. Important social issues must often be decided on the basis of incomplete or uncertain scientific information.

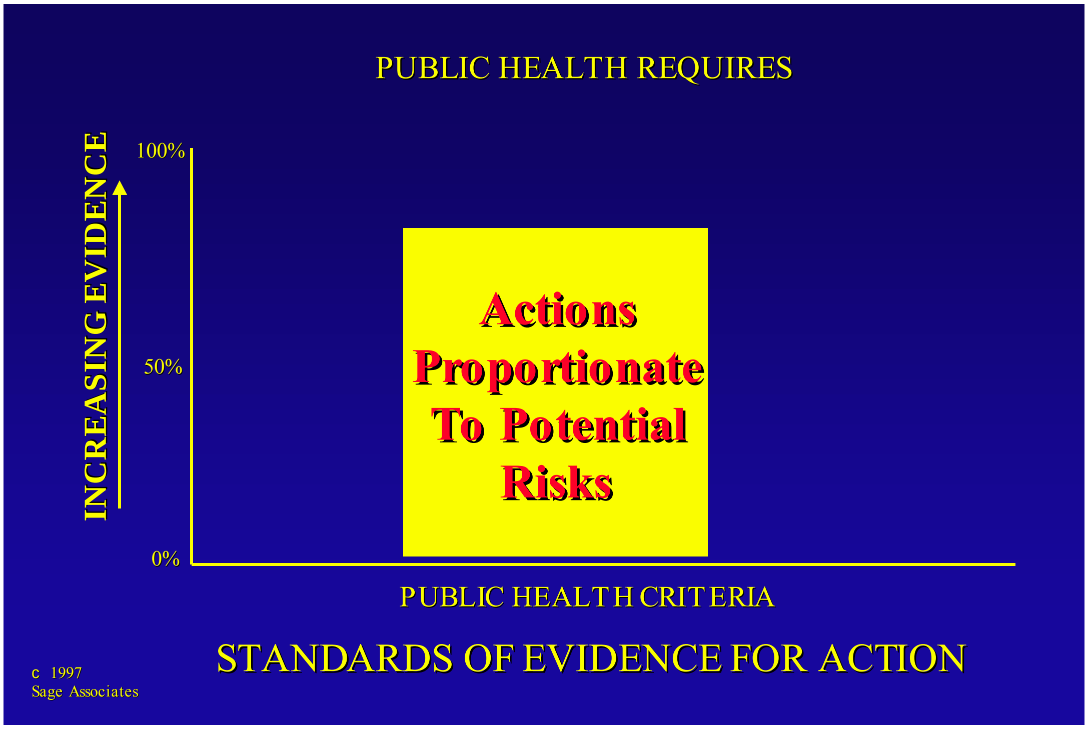
PUBLIC HEALTH REQUIRES
INCREASING EVIDENCE
100%
50%
0%
Actions Proportionate To Potential Risks
PUBLIC HEALTH CRITERIA
STANDARDS OF EVIDENCE FOR ACTION
c 1997
Sage Associates

Figure 2 **Public Health Standard of Evidence for Decisions**

BioInitiative 2012 ©

Biologically-based Exposure Standards for Low-Intensity Electromagnetic Radiation

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